

**Randomized Trial of Activated Marrow Infiltrating Lymphocytes alone  
or in Conjunction with an Allogeneic GM-CSF-based Myeloma Cellular  
Vaccine in the Autologous Transplant Setting in Multiple Myeloma**

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**Protocol History:**

Amendment 1: March 19, 2010  
Amendment 2: August 06, 2010

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## **Trial Synopsis**

**Protocol Title:** Randomized Trial of Activated Marrow Infiltrating Lymphocytes alone or in Conjunction with an Allogeneic GM-CSF-based Myeloma Cellular Vaccine in the Autologous Transplant Setting in Multiple Myeloma

**Patient Population:** Patients with active myeloma (Stage II/III) that have completed induction therapy and are eligible for an autologous stem cell transplant.

**Number of Patients:** Will treat a total of 32 evaluable patients in a 1:1 randomization of aMILs vs aMILs plus vaccine. An evaluable patient is defined as one which has received the activated MILs and is at least 6 months post-transplant.

## **Study Objectives:**

### **Primary:**

- Response rate utilizing Blade' criteria

### **Secondary:**

- Progression-free and overall survival
- **Feasibility**
- **Safety**
- Evaluate anti-tumor immune response
- Evaluate the effect of aMILs on osteoclastogenesis.
- Determine the effect of aMILs on myeloma precursors.

## **Eligibility Criteria:**

### **Inclusion:**

- Durie-Salmon Stage II or III multiple myeloma
- Newly diagnosed receiving treatment or having completed induction therapy.
- Relapsed myeloma not previously transplanted within the past 5 years.
- Measurable serum and/or urine M-protein from *prior to induction therapy* documented and available. A positive serum free light assay is acceptable.
- Age  $\geq 18$  years old
- ECOG performance status of 0 - 2
- Meet all institutional requirements for autologous stem cell transplantation
- The patient must be able to comprehend and have signed the informed consent

### **Exclusion:**

- Diagnosis of any of the following plasma cell disorders:

- POEMS syndrome (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein [M-protein] and skin changes)
  - Non-secretory myeloma (no measurable protein on Serum Free Lite Assay)
  - Plasma cell leukemia
  - Amyloidosis
- Use of corticosteroids (glucocorticoids) within 21 days of pre-transplant vaccine or bone marrow collection
- Use of any myeloma-specific therapy other than lenalidomide within 21 days of pre-transplant vaccine.
- Infection requiring treatment with antibiotics, antifungal, or antiviral agents within seven days of vaccination or bone marrow collection
- Participation in any clinical trial, within four weeks prior to vaccination or bone marrow collection on this trial, which involved an investigational drug or device
- History of malignancy other than multiple myeloma within five years of vaccination or bone marrow collection, except adequately treated basal or squamous cell skin cancer.
- Active autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus) requiring systemic treatment. Hypothyroidism without evidence of Grave's Disease or Hashimoto's thyroiditis is permitted.
- Evidence of spinal cord compression at time of transplant
- Positive for HTLV 1 and 2 infection

## Table of Contents

<b>Trial Synopsis .....</b>	<b>2</b>
<b>1 Study Overview .....</b>	<b>8</b>
<b>2 Objectives of the Study .....</b>	<b>10</b>
<b>2.1 Primary Objective .....</b>	<b>10</b>
2.1.1 Evaluate Response Rates utilizing the Blade' criteria .....	10
<b>2.2 Secondary Objectives .....</b>	<b>10</b>
2.2.1 Evaluate Progression-free Survival and Overall Survival .....	10
2.2.2 Feasibility of clinical design .....	10
2.2.3 Safety .....	10
2.2.4 Determine Tumor-specific Responses .....	10
2.2.5 Effect of aMILs on Bone Metabolism .....	10
2.2.6 Effect of aMILs on Myeloma clonogenic precursors .....	11
<b>3 Background .....</b>	<b>11</b>
3.1 Multiple Myeloma .....	11
3.2 Current Therapies for Multiple Myeloma .....	12
3.3 Overview of Cell-mediated Immunity .....	13
3.4 Immune Defects in Patients with Multiple Myeloma .....	13
3.5 Rationale for Immunotherapy of Multiple Myeloma .....	14
3.6 Rationale for the Use of CD3xCD28 Bead-Activated T Cells .....	15
3.7 Clinical Data Using CD3xCD28 Bead-Activated T Cells .....	16
3.8 Rationale for the Use of Activated MILs in Myeloma .....	17
<b>4 Rationale for Study Design .....</b>	<b>22</b>
4.1 Patient Eligibility Criteria for Marrow Collection .....	22
4.2 Eligibility Criteria for Autologous Stem Cell Transplantation .....	24
4.3 Data Collected from Diagnosis, Prior to Induction Therapy .....	24
<b>5 Baseline Evaluations .....</b>	<b>25</b>
<b>6 Randomization .....</b>	<b>26</b>
<b>7 Vaccination .....</b>	<b>26</b>
7.1 Allogeneic Myeloma Vaccine .....	27
<b>8 MILs Collection .....</b>	<b>27</b>
8.1 Timing of MILs Collection .....	27
8.2 MILs Collection Procedure .....	28
<b>9 MILs Activation Process and Product Formulation .....</b>	<b>28</b>
9.1 MILs Activation Process .....	28
9.2 Formulation Storage of Activated MILs T Cells .....	28
<b>10 Mobilization and Collection of Peripheral Blood Stem Cells .....</b>	<b>28</b>
10.1 Mobilization Regimen .....	28

10.2	Peripheral Blood Stem Cell Collection.....	29
10.3	Nomenclature for Numbering of Days .....	29
10.4	Day –2 and Day –1: Melphalan .....	29
10.5	Autologous Stem Cell Transplant (Day 0).....	29
10.6	Day 1 and Day 2 .....	29
10.7	Supportive Care .....	30
10.8	Post-Transplant Filgrastim.....	30
<b>11</b>	<b>Administration of Activated MILs (Day 3) .....</b>	<b>30</b>
11.1	Timing of Infusion of Activated MILs (aMILs).....	30
11.2	Evaluations Prior to Activated MILs T Cell Infusion.....	30
11.3	Pre-Medication Prior to Infusion of Activated MILs .....	31
11.4	Procedure for Administering Activated MILs .....	31
11.5	Safety Monitoring During Administration of Activated MILs.....	31
<b>12</b>	<b>Post-transplant Vaccinations (days 21, 60, 180, 300).....</b>	<b>32</b>
<b>13</b>	<b>Observation and Evaluation After Treatment with Activated MILs .....</b>	<b>34</b>
13.1	Evaluations From Day 4 Through Day 28 Post Transplant.....	34
13.2	Evaluations from Day 60-360 Post Transplant.....	35
13.3	Management of Progressive Disease .....	36
13.4	Discontinuation of Evaluations after Treatment .....	36
13.5	Contraindicated Medications .....	37
<b>14</b>	<b>Risk and Toxicity Assessment.....</b>	<b>38</b>
14.1	Risks of Venous Access.....	38
14.2	Risks of MILs Bone Marrow Collection .....	38
14.3	Potential Microbial Contamination of the Activated MILs .....	38
14.4	Potential Toxicity of Storage Solutions .....	39
14.5	Potential Adverse Effects Associated with the Allogeneic Myeloma Vaccine .....	40
14.6	Potential Adverse Effects Associated with Activated MILs.....	40
14.7	Potential Adverse Events Associated with Autologous Stem Cell Transplant .....	41
14.7.1	Peripheral Blood Stem Cell Mobilization Chemotherapy Regimen...	41
14.7.2	Administration of Filgrastim (G-CSF).....	41
14.7.3	Melphalan .....	41
14.7.4	Leukapheresis .....	41
14.7.5	Hematopoietic Stem Cell Infusion.....	42
14.8	Risks of Interactions Between Activated MILs, Tumor Vaccination & Autologous Stem Cell Transplantation.....	42
<b>15</b>	<b>Adverse Events .....</b>	<b>43</b>
15.1	Case Report Form Reporting .....	42
15.2	Grading of Adverse Events and Toxicities .....	43
15.3	Attribution of Causality .....	43
15.4	Adverse Events Requiring Immediate Notification of IRB, IBC, FDA and NIH RAC .....	44

15.4.2	Investigator Reporting Responsibilities .....	45
15.4.3	Report of Adverse Events to the Institutional Review Board and Institutional Biosafety Committee.....	45
15.4.4	Investigator Reporting to the FDA and RAC .....	45
15.5	Definitions of Response (Blade' Criteria) .....	47
15.6	Survival Endpoints.....	50
15.7	Definitions of Engraftment and Graft Failure.....	50
<b>16</b>	<b>Statistical Considerations .....</b>	<b>51</b>
16.1	Sample Size.....	51
16.2	Statistical Analyses/ Secondary objectives .....	52
<b>17</b>	<b>Ethical, Regulatory, and Administrative Considerations .....</b>	<b>54</b>
17.1	Informed Consent.....	54
17.2	Institutional Review .....	54
17.3	Tissue Use for Research Purposes .....	54
<b>18</b>	<b>Study Monitoring and Data Collection .....</b>	<b>55</b>
18.1	Study Monitoring .....	55
18.1.1	Completion of Case Report Forms (CRFs).....	55
18.1.2	Cell Therapy Laboratory (CTL) Cell-Processing Facility .....	55
18.2	Maintenance of Study Documentation.....	56
18.2.1	Retention of Records.....	56
18.3	Final Study Report .....	56
18.4	Investigational Product Labeling and Accountability.....	56
18.4.1	Investigational Product Labeling .....	56
18.4.2	Investigational Product Accountability.....	57
<b>19</b>	<b>References .....</b>	<b>58</b>
	<b>Appendix .....</b>	<b>61</b>

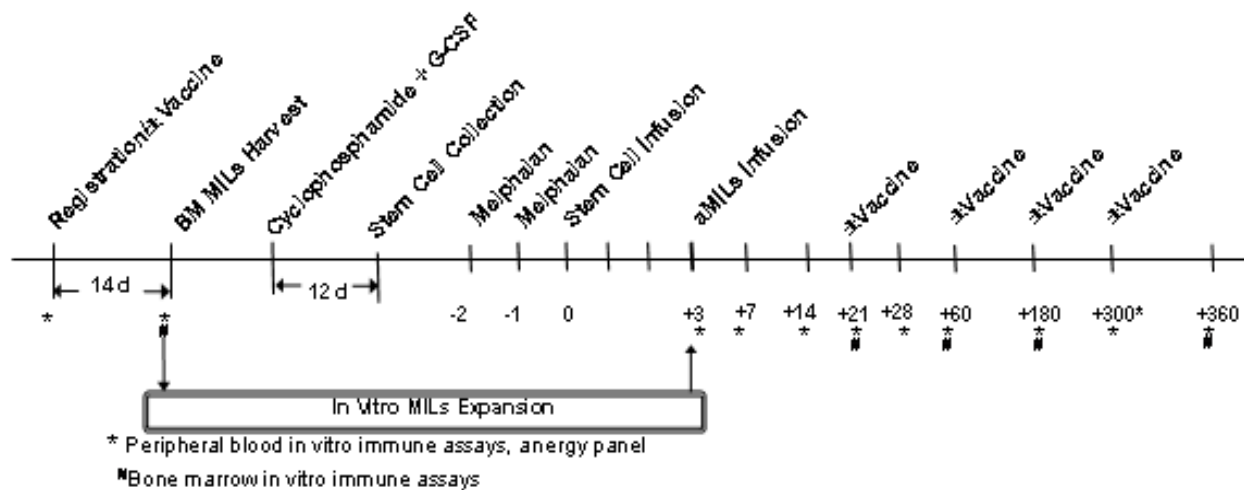
## **1 Study Overview**

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This Phase II randomized clinical study is designed to examine the efficacy of anti-CD3/CD28 activated marrow infiltrating lymphocytes (aMILs) alone or in combination with an allogeneic myeloma, GM-CSF vaccine in study subjects undergoing an autologous stem cell transplant for the treatment of multiple myeloma. 16 patients will be treated with aMILs alone and 16 with aMILs plus the vaccine.

Patients will undergo a bone marrow aspiration to obtain marrow infiltrating lymphocytes (MILs) that will be used to produce aMILs. The MILs can either be obtained at diagnosis prior to the initiation of induction treatment or upon completion of induction therapy. MILs are collected by bone marrow aspiration of ~200ml of marrow. This product will be used to expand the aMILs. During the *in vitro* expansion process, T cells will be activated and *ex vivo* expanded by co-stimulation with anti-CD3 and anti-CD28 monoclonal antibodies covalently attached to super-paramagnetic microbeads. Patients will be treated with a standard high-dose chemotherapy regimen for multiple myeloma consisting of single agent melphalan (200mg/m<sup>2</sup>). Patients will then receive their stem cells. Three days (Day 3) following stem cell infusion, patients will receive a single infusion aMILs. Because of the potential negative impact of G-CSF on T cell trafficking to the bone marrow, patients will not receive post-transplant G-CSF. For patients assigned to the vaccine arm, the first vaccine will be administered 2 weeks prior to the bone marrow collection and the post-transplant vaccines will be administered on days 21, 60, 180 and 300. The vaccine will consist of two irradiated allogeneic myeloma cell lines, H929 and U266 admixed with K562/GM-CSF.

Disease response as determined by the Blade' criteria will be the primary endpoint of the trial at one year. Additional study endpoints include progression free survival, parameters of T cell reconstitution, anti-tumor immune responses as well as the effect on osteoclastogenesis and clonogenic myeloma precursor cells.

**Figure 1.1: Study Schema**



## **2 Objectives of the Study**

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### **2.1 Primary Objective**

Evaluate the clinical efficacy of activated marrow infiltrating lymphocytes (aMILs) administered alone or in combination with an allogeneic myeloma cell vaccine combined with a GM-CSF producing bystander cell in patients undergoing an autologous stem cell transplantation setting for multiple myeloma.

#### **2.1.1 Evaluate Response Rates utilizing the Blade' criteria**

- Complete Response (CR) rate
- Near Complete Response (nCR) rate
- Very Good Partial Response (VGPR) rate
- Partial Response (PR) rate
- Minimal Response (MR) rate
- Overall response rate (CR, VGPR, PR)

### **2.2 Secondary Objectives**

#### **2.2.1 Evaluate Progression-free Survival and Overall Survival**

Patients will be monitored for progression/relapse on Days 60, 180, and 360, and as clinically indicated. Following one year follow-up, patients will be followed annually for the next four years.

#### **2.2.2 Feasibility of clinical design**

- Patient accrual
- Adherence to study schema
- Protocol violations
- Drop-out rate

#### **2.2.3 Safety**

- Treatment-related mortality
- Grade 3 or greater hematologic toxicity

#### **2.2.4 Determine Tumor-specific Responses**

- Evaluate tumor specific responses in blood and bone marrow
- Examine T cell responses to DC-pulsed myeloma cell lines
- Examine induction of novel antibody responses

#### **2.2.5 Effect of aMILs on Bone Metabolism**

Parameters of bone turnover that will include:

- RANKL/OPG ratio
- Serum C Telopeptide levels
- bAlkaline phosphatase and osteocalcin

#### 2.2.6 Effect of aMILs on Myeloma clonogenic precursors

- Examine side population of CD19 enriched PBLs throughout study.

## 3 Background

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### 3.1 Multiple Myeloma

Multiple myeloma is a plasma cell dyscrasia that is the most common cancer of the bone marrow.[1, 2] In 2008, the estimated incidence of myeloma in United States was 19,920.

Multiple myeloma is most often diagnosed in middle aged and elderly individuals. The most common sites of disease are the bone and bone marrow. Malignant plasma cells arise from clonal expansion and accumulate in the bone marrow in masses known as plasmacytomas. These plasma cells produce large amounts of monoclonal immunoglobulins, most commonly IgG (50-60%) and IgA (20-25%) and occasionally IgD, IgM and IgE. [3]Patients often suffer from bone pain and skeletal fragility.[4] Plasmacytomas are osteolytic in nature and often confined to the central skeleton, skull, and femur. Bone destruction is usually localized but can be present throughout the skeleton.[5]

The etiology remains unknown, but risk factors are thought to include chronic immune stimulation, autoimmune disorders, exposure to ionizing radiation, occupational exposure to pesticides or herbicides, occupational exposure to dioxin, and perhaps prolonged use of certain hair coloring products. [6, 7]

The diagnosis is made using several criteria including results of radiographic skeletal survey, bone marrow examination, and measurement of serum and/or urine monoclonal protein (M-protein).[8, 9] Patients are also classified by stage (Stage I, II, III) according to the status of bone lesions, hemoglobin, serum calcium,  $\beta$ -2 microglobulin, C-reactive protein and M-protein.[10] The M-protein is often used to monitor response to treatment via measurement of serum and/or urine analysis of Bence-Jones protein, the light chain component of the M-protein.

Multiple myeloma is a largely incurable disorder and most patients will die of their disease. A variety of chemotherapy agents have been used to treat the

disease but few patients experience long-term disease-free survival with current therapeutic approaches. The median overall survival for patients is approximately three years.[11, 12] There is an urgent need for more effective therapies to treat this challenging disease.

### **3.2 Current Therapies for Multiple Myeloma**

Treatment for multiple myeloma is dependent on the stage of the disease. Patients with Stage I disease are often monitored without treatment. Patients with Stage II and III disease are usually treated with chemotherapy until a response is achieved. For many years, conventional therapy has been the combination of melphalan and prednisone, which achieves an overall response in most patients. Approximately 40% of patients demonstrate a greater than 75% reduction in the M-protein.[13] However, nearly all patients will eventually fail this treatment and the median overall survival is only 3 years with this therapy.[13] A variety of different combination chemotherapy regimens have been developed in an attempt to improve on these results. Unfortunately, regardless of the type of initial treatment, the disease will recur and the 5-year survival is less than 30%.[13, 14]

Dose intensification using high-dose chemotherapy followed by autologous stem cell transplantation has recently been used to increase the response rate and improve the outcome of patients with multiple myeloma.[15-26] In 1996, the French Myeloma Intergroup reported the results of a randomized clinical trial, which compared high-dose chemotherapy supported by autologous bone marrow support to conventional chemotherapy in patients with previously untreated multiple myeloma.[22] The study demonstrated the superiority of high-dose therapy in terms of response rate, event-free survival and overall patient survival. This pivotal study has led to the widespread use of high-dose chemotherapy with autologous stem cell support as standard of care in multiple myeloma patients with good performance status.

The Southwest Oncology Group study of 72 patients with chemotherapy-refractory multiple myeloma treated with high- dose melphalan (200 mg/m<sup>2</sup>) followed by peripheral blood stem cell support was recently reported.[15](16) The regimen was well tolerated resulting in an overall response rate of 65% with approximately 30% of patients achieving complete remission. The median progression-free survival was 11 months and the median overall survival was 19 months. These clinical results compare favorably to studies in this patient population from other clinical investigators.

In the allogeneic transplant setting, long-term responses have been demonstrated. However, this treatment is associated with severe graft versus host disease (GVHD) and substantial mortality, which has limited its use.[27]

A major goal of newer studies has been to increase the overall efficacy of autologous stem cell transplantation without added toxicity. Clearly, the ability to impart a myeloma-specific immune response without the toxicity seen with allogeneic transplants offers significant appeal. Recent studies attempting to utilize vaccine approaches alone or in combination with adoptive immunotherapy have shed significant light into the potential efficacy of these approaches. More importantly, these studies underscore the profound limitations of the current interventions and enabled the development of novel strategies with greater anti-tumor specificity.

### **3.3 Overview of Cell-mediated Immunity**

The human immune system is made up of many kinds of cells that are responsible for eliminating harmful invaders such as viruses or cancer from the body. One type of lymphocyte, the T cell, plays a central role in orchestrating most immune responses. T cells become activated when they recognize antigens, specific elements of microbes or tumor cells, as foreign to the body. This occurs when antigens are taken up, processed and presented by an antigen-presenting cell (APC) to a molecular complex on the T cell. This molecular complex contains the T cell receptor (TCR) associated with the CD3 signaling complex. [27]

The primary signal for activating a T cell takes place when the TCR expressed on its surface binds to a processed antigen present on the surface of an APC. Each individual T cell only expresses a single TCR capable of recognizing a specific antigen. However, the many billions of T cells found in the human body express millions of different TCRs, thereby enabling recognition of millions of distinct antigens. Only a specific T cell that recognizes a particular antigen will become activated during a normal immune response.

APCs must deliver a second signal in order to activate T cells. This co-stimulatory signal occurs when receptors on APCs bind to CD28 receptors on T cells. Activation of T cells takes place when APCs bind to the TCR/CD3 complex and CD28 receptor. These activated T cells are exquisitely sensitive to further stimulation and also secrete a variety of chemical messengers called cytokines. This process further augments the immune response both by driving continued activation and proliferation of T cells and recruiting and stimulating other cells of the immune system. This cascade of events ultimately leads to destruction of pathogens such as tumor cells and viruses.

### **3.4 Immune Defects in Patients with Cancer Including Multiple Myeloma**

The inability of a patient's own immune system to respond to and control cancer may be due to a number of problems. Defects in both the afferent (responding)

as well as efferent (acting) arms of the immune system are well documented in cancer patients. Deficits in the afferent arm of immunity include zeta chain defects in the TCR, which contribute to signaling problems in T cells. In addition, patients with hematological malignancies including chronic lymphocytic leukemia and multiple myeloma demonstrate significant narrowing of the broad spectrum of T cell receptors present in healthy individuals.[28-30] This narrow T cell receptor repertoire may limit the patient's ability to recognize and respond to tumor cells as well as other pathogens. This may contribute not only to cancer progression, but also to the infections that are often observed in patients with hematological malignancies. These defects are both a result of the malignancy itself as well as cytotoxic therapy that can damage T cells. Additionally, ineffective induction of CD40L (CD154) on T cells has been demonstrated in cancer patients.[31] Without CD40L signaling, APCs are not capable of being activated or presenting antigen to T cells. Poor co-stimulation by APCs due to non-responsive elements or defects in the co-stimulatory pathway has been observed in cancer patients.[27] Problems with the effector arm of the immune system in cancer patients include the presence of relatively low numbers of cytotoxic T lymphocytes (CTL), which are required to kill the tumor cells. Additionally, some cancers including multiple myeloma produce cytokines that inhibit the function of normal T cells or APCs.[32] The deficits in immunity may limit the ability of the patients' own T cells to mount an effective immune response to their own cancers.

### **3.5 Rationale for Immunotherapy of Multiple Myeloma**

Immunotherapy is one approach to improving the outcome of patients with multiple myeloma. As noted above, defects in the host's immune system are present in patients with cancer including multiple myeloma. These deficits are thought to play a role in the patient's inability to generate an anti-tumor response and control the disease. A variety of therapeutic approaches are now being developed that stimulate the patient's immune system. Several groups are using idiotype vaccines to stimulate T cell-mediated responses to the patient's tumor cells.[33, 34] Using this approach, patients are typically treated with an autologous transplant followed by vaccination with their own idiotype, which is derived from their unique M-protein. Promising clinical results have been observed in some of these clinical trials. However, many patients have weakened immune systems after the transplant and have been unable to respond to the vaccine[35]

We have recently completed a clinical study utilizing autologous tumor vaccines in the autologous transplant setting. In this study, newly diagnosed patients underwent a bone marrow collection to obtain autologous tumor that was combined with the K562/GM-CSF producing bystander cell line in the final vaccine formulation [36]. Patients were administered the vaccine pre-transplant

in an effort to prime tumor-specific T cell responses *in vivo*. The lymphocytes were then collected and infused at the time of transplantation in an attempt to impart an early anti-tumor effect. The post-transplant vaccinations started 6 weeks post-transplant and were administered every 3 weeks for a total of 8 post-transplant vaccines. The rationale for starting vaccinations this early post-transplant is based on murine data demonstrating the existence of an early endogenous tumor-specific lymphocyte expansion that can then be maintained with vaccinations in the post-transplant setting [37]. While this study showed evidence of the generation of tumor-specific T cell responses, the degree of lymphopenia post-transplantation was greater than initially predicted with absolute CD4 numbers considerably below normal up to one year post-transplant. One attempt to increase vaccine efficacy is the ability to enhance T cell reconstitution and utilize vaccines at a time when maximal T cell responsiveness can be guaranteed.

Recently, several investigators have documented the potent anti-tumor effects of donor lymphocyte infusions (DLI) administered to patients who relapse after allogeneic stem cell transplantation.[38-42] Unfortunately, a high incidence of GVHD has been observed with DLI, which has significantly limited its therapeutic application.

Investigators have documented that T cells with anti-tumor activity are present in the blood of patients with multiple myeloma.[43] If sufficient numbers of the patient's own T cells could be activated and expanded, they could be used in combination with an autologous stem cell transplant. This would provide a potentially safer therapeutic alternative to DLI. Patients would avoid the risks of GVHD as well as the substantial morbidity and mortality (up to 40%) that has been documented in multiple myeloma patients undergoing allogeneic stem cell transplantation. [44]

Additionally, recent clinical data provide further rationale for the administration of autologous T cells in patients undergoing autologous stem cell transplantation. Several clinical studies have documented improved therapeutic outcome in patients with multiple myeloma (as well as non-Hodgkin's lymphoma, breast cancer, and ovarian cancer), who experience more rapid and/or complete recovery of their peripheral blood lymphocytes after autologous stem cell transplantation.[45-47]

### **3.6 Rationale for the Use of CD3xCD28 Bead-Activated T Cells**

Carl June and colleagues developed technology to activate T cells of the immune system outside of the body (*ex vivo*)[48]. This procedure is based on the roles of the CD3 signaling complex and CD28 receptor in the activation of T cells. In the manufacturing process, T cells are stimulated *ex vivo* using

monoclonal antibodies that bind to the CD3 and CD28 molecules expressed on the surface of T cells. The antibodies are attached to microscopic beads, thereby creating artificial APCs. As a result, a universal reagent can be developed to activate all T cells. Single beads, which coordinate CD3 and CD28 signals, optimize T cell activation and allow rapid expansion of T cells. Pre-clinical studies have shown that T cells can be generated *ex vivo* using these beads from patients with human immunodeficiency virus (HIV) or cancer. [49, 50]

Studies have demonstrated that T cells can be activated and expanded more than one hundred fold in less than 10-12 days with anti-CD3/CD28 bead activation with a predominant expansion of CD4 over CD8 T cells. Further studies have shown that the patients' activated T Cells express high levels of CD154 (CD40L), CD137 (4-1BB) and other key effector molecules such as CD134 (OX-40), CD54 (ICAM) as well as important receptors such as CD25 (IL-2 receptor). Finally, this process generates T cells that display a Th1 phenotype secreting high levels of IL-2 and interferon-gamma that are known to play essential roles in activating both helper T cells as well as cytolytic T cells. These features demonstrate the ability to reverse tolerance in cancer patients and restore T cell responsiveness that may enable these T Cells to restore anti-tumor immunity.

### **3.7 Clinical Data Using CD3xCD28 Bead-Activated T Cells**

A number of independent clinical trials have been conducted in which patients have been treated with T cells activated *ex vivo* using a CD3/CD28 bead-based technology. T cells activated in this manner have previously been tested in patients undergoing a autologous stem cell transplant for relapsed or refractory Non-Hodgkin's lymphoma [51]. T cells were collected prior to high-dose chemotherapy. Fourteen days following the peripheral blood stem cell infusion, activated and expanded T cells were administered. Three patients were treated at a median cell dose of  $0.4 \times 10^9$ , twelve patients were treated at a median cell dose of  $1.6 \times 10^9$ , and two patients were treated with a median cell dose of  $9.8 \times 10^9$ . Infusion related toxicities experienced by the two patients at the highest dose level included transient fever, dyspnea, rigors and pulmonary edema. Maximal responses included five patients with complete responses, seven patients with partial responses, and five patients with stable disease.

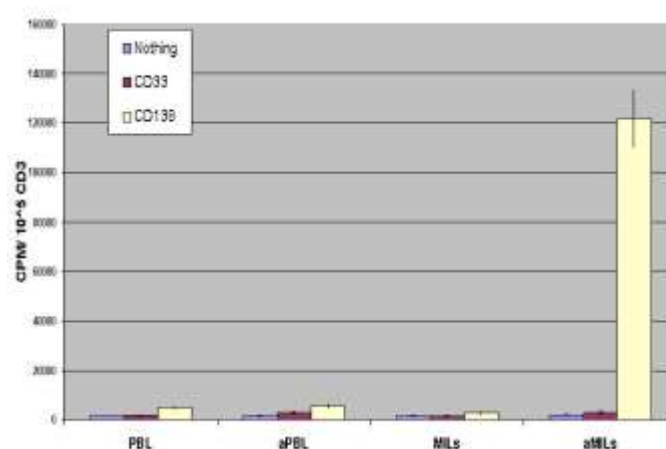
We recently completed a Phase I/II study in multiple myeloma in which 32 patients were administered anti-CD3/CD28 activated T cells. T cells were effectively expanded in all patients with an average fold-increase of 268 ( $\pm$  101). T cells were then infused on day + 3 following an autologous stem cell transplant. Interestingly, in addition to the *in vitro* expansion, patients experienced an additional *in vivo* expansion reaching maximal expansion on

day +21 that far exceeded the T cell numbers seen in a non-transplanted healthy individual. However, despite the feasibility, safety and evidence of both in vitro and in vivo expansion, the overall clinical response showed CR 6%, PR 34% with an overall response rate of 40% which is no better than standard autologous stem cell transplants. There were no significant toxicities related to the infusion of activated T cells and the majority of adverse events related to T-cells were mild in severity and included fever (19%), chills (17%), asthenia (10%), headache (10%), and nausea (10%).

### 3.8 Rationale for the Use of Activated MILs in Myeloma

#### aMILS have significant anti-myeloma activity

From the above mentioned clinical studies, we have shown the ability of this



**Figure 1: aMILs exhibit marked anti-myeloma activity.**  
T cells from blood or marrow were either activated with anti-CD3/CD28 beads or left unstimulated. Tumor specificity was determined by incubating the cells either with autologous plasma cells (CD138), autologous non-malignant myeloid cells (CD33) or nothing and determining H-thymidine incorporation

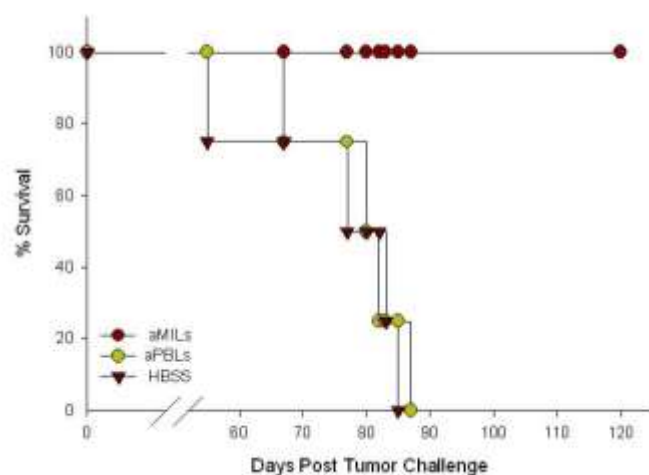
technology to effectively overcome the inherent unresponsiveness seen in T cell from tumor-bearing hosts and to expand upon anti-CD3/CD28 stimulation. This data also underscores a significant limitation of the polyclonal expansion that lacks antigen specificity. To this effect, we have attempted to develop strategies aimed at increasing the tumor specificity of this approach. Specifically,

we have discovered that marrow infiltrating lymphocytes can be effectively activated and expanded with properties suggestive of an effector/memory population. More importantly, they possess several critical features required for effective anti-tumor adoptive immunotherapy: 1) they can be activated and expanded to reasonable numbers; 2) they demonstrate significant specificity against mature plasma cells. PBLs or MILs were activated for 5 days in vitro and tumor specificity was assessed by determining their proliferative response to autologous tumor. As shown in **Figure 1**, whereas activated PBLs (aPBLs) failed to show measurable tumor specificity, activated MILs (aMILs) exhibited marked tumor reactivity. Interestingly, no reactivity was appreciated against



normal hematopoietic elements. Interestingly, in addition to their significant activity against mature plasma cells, aMILs were also capable of significantly limiting the outgrowth of clonogenic myeloma precursors suggesting a broad range of tumor antigen recognition.

Another critical aspect of effective adoptive immunotherapy is the ability of T cells to traffic to the tumor microenvironment. SDF-1 (stromal derived factor - 1) and its cognate chemokine receptor, CXCR4 are critical factors in cell trafficking in the marrow. We have shown that a significantly higher percentage of MILs express CXCR4 as compared to PBLs thus increasing the likelihood of trafficking of these cells to the appropriate compartment. [52]. To confirm this, we performed an experiment utilizing NOD/SCID mice. Mice were irradiated and challenged with the H929 myeloma cell line and then 18 days later either given activated MILs, activated PBLs or no T cells. The T cell



doses used corresponded to doses ranging from  $3 - 20 \times 10^7$  CD3/kg (doses easily achievable in humans). As shown in **Figure 2**, activated PBLs imparted no measurable anti-tumor effect compared to no T cells whereas the mice receiving activated MILs demonstrated 100% survival with no evidence of detectable tumor. Furthermore, T cells were detectable in the marrows of mice having received aMILs whereas no T cells but CD138+ plasma cells were seen in the bone marrows of mice treated with HBSS or

aPBLs. These findings confirm our in vitro data and suggest the overall efficacy of this approach.

### **aMILs inhibit plasma cell colony outgrowth of myeloma progenitors**

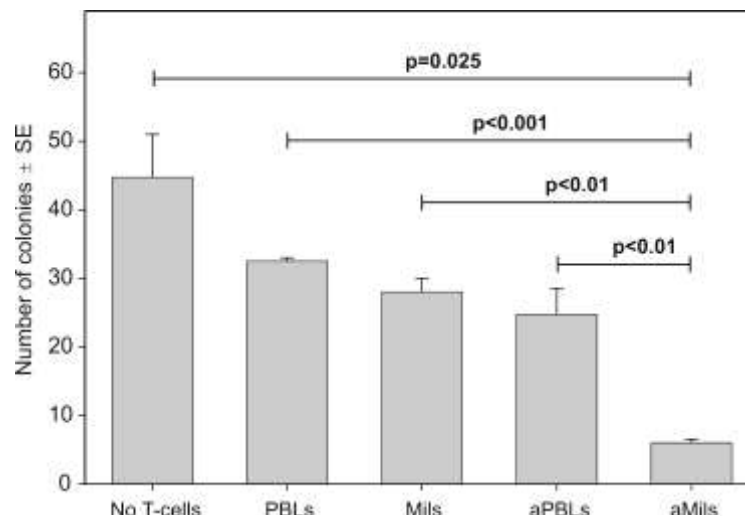
The CD138+ plasma cell represents a terminally differentiated cell with minimal self-renewal potential.

**Fig 2: aMILs confer significant tumor-free survival advantage.** NOD/SCID mice were challenged with the human myeloma cell line, H929. 18 days later they either received HBSS, aPBLs or aMILs at doses ranging from  $1-5 \times 10^6$  /mouse and were followed for tumor-free survival.

As such, it unlikely represents the clonogenic precursor of the malignant myeloma

cell. Complete eradication of mature plasma cells will seldomly result in sustained remissions. When marrow from myeloma patients is depleted of CD34+ hematopoietic stem cells as well as CD138+ plasma cells and CD3+ T cells and plated on methylcellulose, there is an outgrowth of CD138+ plasma cells with the same light chain restriction of the initial myeloma clone. Early evidence that infiltrating T cells may mediate an anti-MM effect was provided by the observation that the number of MM colonies arising in the clonogenic assay were significantly increased if T cells were first removed from the marrow cultures prior to plating. With this knowledge and our data demonstrating a greater memory/effector phenotype of MILs over PBLs, we compared the number of colonies arising from CD34/CD138 marrow cells depleted of T cells to those seen when the same marrow precursors were co-incubated with either resting or previously activated PBLs or MILs. These studies showed a clear reduction of MM colony growth upon co-incubation with T cells. Interestingly, while aMILs exerted the most potent effect on inhibiting plasma cell colony outgrowth, even the add-back of purified, unstimulated MILs demonstrated measurable anti-tumor efficacy (**Fig 3**). It should be noted that CFU-GM colony outgrowth was unaltered with the addition of aMILs suggesting the myeloma specificity of aMILs with no reactivity towards normal hematopoietic elements. These results demonstrate the ability of *in vitro* anti-CD3/CD28 stimulation to enhance antigen recognition and are consistent with the hypothesis that T cell recognition by aMILs occurs not only against antigens expressed on CD138+ plasma cells, but also on their progenitors – an observation that if true will significantly enhance the therapeutic efficacy of these cells.

In summary, several conclusions can be drawn from this data that make adoptive therapy utilizing aMILs an approach worthy of pursuing clinically: 1) the tumor



**Fig 3: Inhibition of Clonogenic Outgrowth.** Bone marrow from myeloma patients was depleted of CD34+, CD138+ plasma cells, and CD3+ cells, resuspended in methylcellulose overnight and then plated out and scored for the development of CD138+ colonies cultured in the presence of various T cell populations.

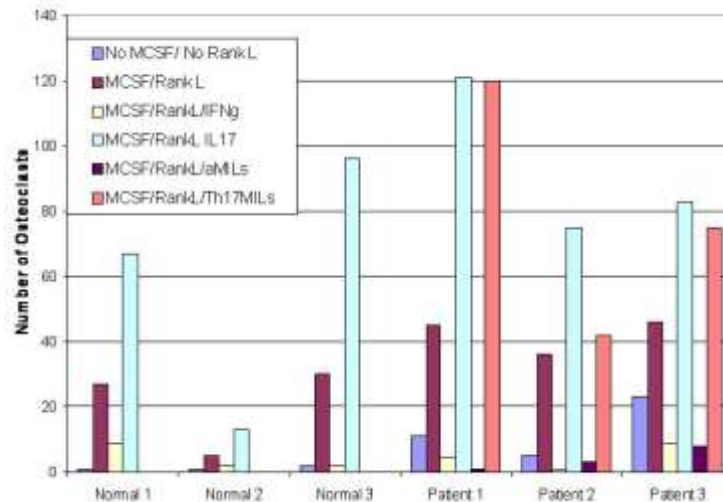
micro-environment of myeloma patients contains lymphocytes (MILs) with greater anti-tumor specificity than is observed in peripheral lymphocytes; 2) the kinetics of T cell activation of MILs compared to PBLs is consistent with expansion of a memory T cell population; 3) anti-CD3/CD28 beads are capable of reversing tolerance and restoring T cell responsiveness to

tumor-specific antigens; 4) the tumor specificity of aMILs greatly exceeds that of aPBLs; 5) aMILs offer the prospect of significantly impairing osteoclastogenesis; and 6) MILs exhibit specificity towards a broad range of antigens present on both mature plasma cells as well as their clonogenic precursors thus offering the theoretical possibility of eradicating the disease.

### **aMILs inhibit osteoclastogenesis**

CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Tregs) are responsible for many aspects of immune regulation which limit responses to auto-antigens in preventing autoimmunity, to allo-antigens in transplantation tolerance, and even pregnancy. In the tumor-bearing host, this regulatory T cell population plays a dominant role in suppressing tumor specific immunity and is likely key to the induction of tumor antigen associated tolerance. We have shown that the marrow of myeloma patients fails to contain Tregs - a finding with significant implications for adoptive T cell transfer.

In myeloma, both the tumor and the stroma are a major source of IL-6 which in combination with TGF $\beta$  induces an effector Th17 phenotype [53]. Recent evidence has suggested that a reciprocal relationship exists between Tregs and Th17 cells[54]. A significant amount of literature has recently emerged highlighting the role of Th17 cells in autoimmune processes including



**Fig 4: The Th17 phenotype of myeloma MILs induces osteoclastogenesis.** BM from normal or myeloma patients was incubated with M-CSF/Rank-L alone or with the addition of IL-17, Th17 MILs,  $\gamma$ IFN, or activate MILs. Osteoclasts were scored on day 21.

experimental autoimmune encephalitis, inflammatory bowel disease and rheumatoid arthritis. In attempting to understand the function of Th17 effector cells within the myelomatous bone marrow, we examined the role of MILs on osteoclastogenesis whose activation is responsible for the lytic bone lesions seen in this disease. Osteoclasts were generated from bone marrow of normal donors or myeloma patients in the presence of M-CSF and Rank-L. As shown in **Fig 4**, the addition of either IL-17 or Th17-skewed MILs significantly increased the number of osteoclasts generated whereas, the addition of  $\gamma$ IFN dramatically reduced these numbers. Interestingly, co-culture of osteoclast precursors with aMILs ( $\gamma$ IFN producing Th1 cells) reduced osteoclast formation to a similar degree as  $\gamma$ IFN alone. Taken together, these findings provide a biologic rationale as to the genesis and function of Th17 MILs in the myelomatous bone marrow and also explain the absence of Tregs in this disease as compared to normal marrow. Furthermore, in addition to anti-myeloma activity of aMILs, they may also play a significant role in limiting osteoclast-induced bone destruction - a prospect that could have considerable therapeutic impact and justify the rationale for utilizing autologous marrow infiltrating lymphocytes as a means to augment myeloma-specific anti-tumor immunity.

## 4 Rationale for Study Design

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Current treatments for relapsed or refractory multiple myeloma are unsatisfactory. Conventional therapy using alkylating agents as well as the newer agents such as bortezomib, thalidomide or lenalidomide can generate responses, but disease recurrence and progression are inevitable. More aggressive treatment regimens incorporating high-dose chemotherapy with stem cell support have led to increased response rates and improved survival. Nevertheless, only about 30% of patients achieve complete remissions [20] and few patients achieve durable remissions with this form of therapy. There is increasing interest in using immunotherapeutic approaches in combination with high-dose chemotherapy and autologous stem cell transplantation to improve upon these results.

Utilizing peripheral blood lymphocytes in a previously conducted clinical trial, T cells were reproducibly generated from all patients. Delivery of activated T cells in the transplant setting is attractive in light of data from both animal and human studies demonstrating the enhanced activity of T cells when administered in a lymphopenic setting [37, 55]. Furthermore, clinical data suggests that patients experiencing more rapid recovery of their peripheral blood lymphocytes after autologous stem cell transplantation have improved survival. [45-47] Based on these data, we propose to evaluate the safety, feasibility and therapeutic activity of aMILs after high-dose chemotherapy and autologous stem cell transplantation in patients with multiple myeloma.

### 4.1 Patient Eligibility Criteria for Marrow Collection

Patients interested in participating on this study will sign consents. Eligibility will subsequently be determined based on the inclusion and exclusion criteria listed below. Please refer to Patient Study Calendar, Appendix E. It should be noted, that bone marrow may be collected prior to the initiation of therapy. MILs will not be expanded until the eligibility for the trial has been determined.

To be eligible, the patient must meet **all** of the following inclusion criteria:

- Previous diagnosis of multiple myeloma based on standard criteria as defined in Appendix A (*Diagnostic Criteria for Multiple Myeloma*). Tests need not be performed within 30 days of vaccination or bone marrow collection.
- Durie-Salmon Stage II or III disease as defined in Appendix B (*Durie Salmon Staging for Multiple Myeloma*) at any time since diagnosis

- Measurable serum and/or urine M-protein from *prior to induction therapy* documented and available. A positive serum free lite assay is acceptable.
- Age  $\geq$  18 years old
- ECOG performance status of 0 -2 (refer to Appendix C)
- Life expectancy  $\geq$  6 months
- Corrected serum calcium  $< 11$  mg/dL, and no evidence of symptomatic hypercalcemia. (Corrected serum calcium is calculated by adding 0.8 mg/dL to the measured serum calcium for every 1 g/dL that the serum albumin falls below 4.0 g/dL.)
- Serum total bilirubin and SGPT (ALT)  $\leq 2.0$  times the upper limit of normal
- Serum creatinine  $< 2.0$  mg/dL
- The patient must be able to comprehend and have signed the informed consent

At the time of consent signing, patients will be **ineligible** for the study if any of the following exclusion criteria apply:

- Diagnosis of any of the following cancers:
  - POEMS syndrome (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein [M-protein] and skin changes)
  - Non-secretory myeloma (no measurable protein on Serum Free Lite Assay)
  - Plasma cell leukemia
- Diagnosis of amyloidosis
- Previous hematopoietic stem cell transplantation within the past 5 years.
- Known history of HIV infection.
- Use of corticosteroids (glucocorticoids) within 21 days of vaccination.
- Use of any myeloma-specific therapy within 21 days of vaccination.
- Infection requiring treatment with antibiotics, antifungal, or antiviral agents within seven days of consent signing

- Participation in any clinical trial, within four weeks prior to vaccination or bone marrow collection on this trial, which involved an investigational drug or device
- History of malignancy other than multiple myeloma within five years of consent signing, except adequately treated basal or squamous cell skin cancer.
- Active autoimmune disease (e.g., rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus) requiring systemic treatment. Hypothyroidism without evidence of Grave's Disease or Hashimoto's thyroiditis is permitted.
- Positive for HTLV1 and/or HTLV2

#### 4.2 Patient Eligibility Criteria for Autologous Stem Cell Transplantation

Prior to transplant, the patients must meet **all** of the following inclusion criteria:

- Myeloma specific therapy with a minimum of 3 cycles.
- Institutional criteria for and has institutional approval to undergo autologous stem cell transplantation as per CP1 and CP2 (<http://www.rig.onc.jhmi.edu/oncres/Protocols/CP1.pdf>; <http://www.rig.onc.jhmi.edu/oncres/Protocols/CP2.pdf>)
- Females of child-bearing potential must have a negative serum  $\beta$ HCG test and be willing to use effective contraception (i.e. a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, or condom with spermicide, or abstinence) up to Day 180.

Prior to transplant, patients will be **ineligible** for the study if any of the following exclusion criteria apply:

- Evidence of spinal cord compression
- Major organ system dysfunction including (but not limited to): New York Heart Association Class III or IV (Appendix D), pulmonary disease requiring the use of inhaled steroids or bronchodilators, renal, hepatic, gastrointestinal, neurologic, or psychiatric dysfunction which would impair patient's ability to participate in the trial
- HIV infection

#### 4.3 Data Collected from Time of Diagnosis, Prior to Induction Therapy

M-protein results from the time of diagnosis, prior to induction therapy, will be recorded, These M-protein results are required for study eligibility, as stated in

Section 4.1, *Patient Inclusion Criteria*. Additional data will be collected when available, but will not be required for entry into the study. This will include, e.g., date of diagnosis, type and duration of induction or other myeloma therapy, as well as data from the time of diagnosis, such as Durie-Salmon stage, beta-2 microglobulin level, albumin, cytogenetics or FISH, bone marrow results, skeletal survey results, and measurement of any known soft tissue plasmacytomas.

## **5 Baseline Evaluations**

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After the patient has been registered in the study, the following evaluations will be performed to determine the patient's baseline status. All tests must be completed within 30 days of the MILs bone marrow collection.

- Complete clinical exam, including complete medical history, review of systems, ECOG performance status, vital signs, weight, physical examination
- Current medications
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin
- CBC with differential and platelet count
- Beta-2 microglobulin
- M-protein, serum:
  - Protein electrophoresis
  - Immunofixation
  - Freelite (sensitive assay for kappa and lambda light chains) only if most recent immunofixation result showed no evidence of M-protein
- M-protein, urine (24 hour collection) for:
  - Total protein
  - Protein electrophoresis
  - Immunofixation
- Quantitative immunoglobulins (IgG, IgM, IgA)
- Study blood (100ml of peripheral blood for research purposes) and 2 green top tubes (for the evaluation of myeloma precursors) Bone



marrow aspirate and biopsy.\* Additional bone marrow sample (10-15 cc) will be obtained for research purposes

- Skeletal survey\*
- Bone turnover markers: Serum C telopeptide levels, bAlkaline phosphatase and osteocalcin

\* Acceptable if performed prior to the autologous stem cell transplant

## **6 Randomization**

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There will be a 1:1 randomization to either aMILs alone or aMILs with the myeloma vaccine. Randomization will be determined based on the following parameters:

1. De novo vs relapsed myeloma
2. International Staging System (ISS) stage 1 or 2 vs. 3
3. Patients in complete remission vs not.

## **7 Vaccination**

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Patients will be administered the allogeneic myeloma cellular vaccine pre- and post-transplantation.

Vaccines will be numbered as follows:

- 1 – pre-transplant vaccine (administered roughly 2 weeks prior to bone marrow collection)
- 2 – d+21 post-transplant (+/- 3 days)
- 3 – d +60 (+/- 10 days)
- 4 – d+180 (+/- 2 weeks)
- 5 – d+300 (+/- 4 weeks)

- CBC with differential will be performed on vaccine days
- Research Blood Collection:

Vaccine arm:

- Patients will have 100ml of blood collected in heparinized syringes at the time of first vaccine administration and another 100 mls on the day of the bone marrow collection. This will enable us to determine the effects of vaccine priming on T cells.
- Patients will have 10 mls of blood collected in tiger top tubes to be used for analysis of GM-CSF serum levels. Blood will be

collected prior to administering the vaccine and 2 days post vaccine at the following vaccine timepoints:

- Vaccine 1 (pre-transplant vaccine)
- Vaccine 2
- Vaccine 4

## **7.1 Allogeneic Myeloma Vaccine**

The allogeneic myeloma vaccine will consist of 3 cells:

H929 – is an unaltered myeloma cell line grown in serum-free medium. This is an IgA cell line isolated from the pleural effusion of a patient with end-stage myeloma. The cell line possesses the t(4;14) as well as the N-13 ras mutation. It also expresses MAGE-A2 and NY-ESO-1.

U266 – is an unaltered myeloma cell line isolated from a patient with plasma cell leukemia. The line contains the t(11;14), overexpression of cyclin D1 and amplification of bcl-2. It also expresses NY-ESO-1 and GAGE-3.

K562/GM-CSF – is an erythroleukemia cell line modified to express stable, high levels of GM-CSF to be used as the GM-CSF-producing bystander. This cell line was chosen in that it lacks HLA surface expression. The cell line produces approximately 1500ng/10<sup>6</sup> cells/24hrs of GM-CSF.

The actual vaccine formulation will consist of 5x10<sup>7</sup> H929 cells, 5x10<sup>7</sup> U266 cells and 5x10<sup>6</sup> K562/GM-CSF cells. All cell lines will be irradiated prior to cryopreservation. Upon thawing, the appropriate volume of each cell line will be mixed and then multiple syringes will be prepared for injection. Five intradermal injections will be administered over 3 limbs to exclude the dominant arm.

The pre-transplant vaccine will be administered 2 weeks prior to the bone marrow collection.

## **8 MILs Collection**

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The bone marrow collection will be performed to obtain the marrow infiltrating lymphocytes (MILs) required for the in vitro activation. The patient's treatment schedule is summarized in Appendix E, *Patient Study Calendar*.

### **8.1 Timing of MILs Collection**

The MILs collection can occur at two separate times. Either at diagnosis prior to the initiation of induction therapy or upon completion of induction therapy.

## **8.2 MILs Collection Procedure**

Each eligible patient will have approximately 200cc of bone marrow collected in syringes containing 2ml of 10,000 units/ml heparin under steady state conditions. The marrow will be collected in 60cc syringes. Sedation and analgesia will be administered per standard institutional practices.

## **9 MILs Activation Process and Product Formulation**

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### **9.1 MILs Activation Process**

The MILs bone marrow product will undergo *ex vivo* activation and expansion of T cells in the Johns Hopkins Cell Therapy Lab (CTL) for up to 10 days. In the activation process, the T cells are activated and expanded by co-stimulation of T cells with anti-CD3 and anti-CD28 antibodies conjugated to super-paramagnetic microbeads (ClinExVivo™ CD3/CD28 Paramagnetic Particles). The super-paramagnetic microbeads are removed at the completion of the process. The product will not be released, unless acceptance criteria have been met.

### **9.2 Formulation Storage of Activated MILs T Cells**

The aMILs products will be formulated in multiple cryopreservation bags. Each bag will contain aMILs formulated in a cryoprotectant consisting of 6% Hetastarch in 0.9% sodium chloride injection supplemented with 2% Human Serum Albumin (HSA) and 5% dimethylsulfoxide (DMSO). The aMILs product will be frozen in a controlled-rate freezer until it reaches  $-80^{\circ}\text{C}$ . The product will then be stored in the vapor phase of a liquid nitrogen freezer at less than  $-135^{\circ}\text{C}$ .

## **10 Mobilization and Collection of Peripheral Blood Stem Cells**

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Following MILs collection, the patient may begin the mobilization regimen, which should be performed according to institutional standards. The autologous stem cell transplant procedures will be carried out as per the institutional standard of care.

### **10.1 Mobilization Regimen**

The mobilization regimen will be cyclophosphamide  $2.5\text{-}3\text{ g/m}^2$ . Filgrastim (Neupogen; G-CSF) will be administered following cyclophosphamide as per institutional standards. Hydration, mesna, and diuretics should be administered with the cyclophosphamide per institutional standards.

## 10.2 Peripheral Blood Stem Cell Collection

Peripheral blood stem cell leukapheresis will be performed approximately 12 days following cyclophosphamide depending on the peripheral CD34<sup>+</sup> cell counts as per standard institutional practice. Leukapheresis will be performed to obtain a minimum of  $2.0 \times 10^6$  CD34<sup>+</sup> cells/kg. Standard leukapheresis will be performed according to institutional guidelines for the collection of peripheral blood stem cells. Up to 2% of the leukapheresis product will be stored for immune assays as long as the removal of these cells does not decrease the number of CD34<sup>+</sup> cells frozen to below  $2.0 \times 10^6$  CD34<sup>+</sup> cells/kg.

For patients failing to mobilize the peripheral stem cells, they will be offered the option of undergoing a standard bone marrow collection.

## 10.3 Nomenclature for Numbering of Days

The day of infusion of the stem cells is called Day 0. Days following autologous stem cell transplant are numbered accordingly, e.g., the first day after transplant is designated as Day 1. Days before the transplant are designated in the negative, e.g., the day prior to transplant is referred to as Day -1.

## 10.4 Day -2 and Day -1: Melphalan

Melphalan will be administered on two consecutive days (Day -2 and Day -1) at a dose of 100 mg/m<sup>2</sup>/day intravenously in sterile water (5mg/ml) over 20-30 minutes.

CBC with differential will be obtained on Day -2 and Day -1 prior to Melphalan infusion.

## 10.5 Autologous Stem Cell Transplant (Day 0)

A CBC with differential and platelets will be obtained prior to stem cell infusion.

A minimum of  $2.0 \times 10^6$  CD34<sup>+</sup> cells/kg of stem cells will be infused. The infusion will occur the day after the last dose of melphalan. Corticosteroids may have an adverse effect on the activity of T cells contained in the stem cell product, and should therefore be avoided. See Section 0, *Contraindicated Medications*.

## 10.6 Day 1 and Day 2

A CBC with differential and platelet count will be obtained on Day 1 and Day 2.

## **10.7 Supportive Care**

All patients will receive supportive care according to institutional clinical guidelines for autologous stem cell transplantation. This may include but not be limited to allopurinol, menstrual suppression, prophylactic antibiotics, empiric antibiotics, intravenous immunoglobulin (IVIG), transfusion of blood products, hyperalimentation, and erythropoietin.

## **10.8 Post-Transplant Filgrastim**

Post-transplant filgrastim (Neupogen; G-CSF) will NOT be permitted on this trial because of the known ability to downregulate CXCR4 which may negatively impact on the ability of aMILs to traffic to the bone marrow following infusion.

## **11 Administration of Activated MILs (Day 3)**

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### **11.1 Timing of Infusion of Activated MILs (aMILs)**

Activated MILs infusions will be scheduled for three days (Day 3) after the administration of the stem cell product. Infusion may be delayed if required by cell processing issues or if warranted by the patient's clinical condition.

### **11.2 Evaluations Prior to Activated MILs T Cell Infusion**

The following evaluations will be made prior to infusion of activated MILs:

- Clinical assessment: review of systems, vital signs
- CBC with differential and platelet count
- Blood in a heparinized green top tube.
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin
- Concurrent medication documentation
- Adverse event documentation

**aMILs will not be infused if:**

- **Temperature >38.5°C**
- **Oxygen saturation < 90% on room air**

- **Patient is felt to be clinically unstable by the medical team**

**If aMILs infusion is delayed because of these issue, the cells will be infused after at least 24 hours from when the issues have resolved.**

### **11.3 Pre-Medication Prior to Infusion of Activated MILs**

The patient will be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or IV, no greater than 30 minutes prior to the infusion of activated MILs. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have a high fever not relieved by acetaminophen.

Intravenous diphenhydramine hydrochloride, epinephrine, hydrocortisone and supplemental oxygen will be available at the patient's bedside in the event of anaphylaxis or an infusion reaction.

Other medications may also be prescribed to treat additional side effects during the course of treatment. However, there are some medications and therapies that may have adverse effects on the activity of activated MILs T Cell therapy and therefore are contraindicated (see Section 0, *Contraindicated Medications*). **In particular, patients should not receive systemic corticosteroids** such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on activated MILs. If steroids are required for an acute infusion reaction, an initial dose of hydrocortisone 100 mg is recommended.

### **11.4 Procedure for Administering Activated MILs**

For the administering of the activated MILs, the patient will be hydrated with D5½NS at approximately 200 ml per hour for at least one hour. If the activated MILs product appears to have a damaged or leaking bag, or otherwise to be compromised, it will not be infused. Otherwise the activated MILs will be thawed and infused through standard blood tubing **without an additional filter** into a peripheral or central IV site. Each of the bags will be infused at a rate of approximately 10 ml per minute. Following aMILs infusion, the patient will be hydrated with D5W½NS at approximately 200 ml per hour for two hours.

### **11.5 Safety Monitoring During Administration of Activated MILs**

During the infusion of activated MILs, the patient's blood pressure, heart rate, respiratory rate, temperature, and peripheral oxygen saturation will be monitored approximately as frequently as the following time intervals:

- Immediately prior to infusion of first bag of activated MILs
- 15 minutes after initiation of first activated MILs
- 30 minutes after initiation of first activated MILs
- 1 hour after initiation of first activated MILs
- 2 hours after initiation of first activated MILs

In the event of a severe allergic reaction (e.g., anaphylaxis, bronchospasm, hypotension) or other serious adverse reaction (i.e., cardiac failure, respiratory failure, severe nausea and vomiting) to the activated MILs the infusion of the activated MILs will be stopped immediately and the patient will be provided with the supportive care deemed necessary by the medical staff at the clinical site. The reaction should be noted as a serious adverse event, and the IRB should be notified within 24 hours.

## **12 Post-transplant Vaccinations (days 21, 60, 180, 300)**

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The allogeneic myeloma vaccine will be administered on day +21, 60 ( $\pm 7$  days), 180 ( $\pm 14$  days) and 300 ( $\pm 14$  days) post-transplant assuming that patients show no evidence of ongoing transplant related toxicity.

The day 21 post-transplant vaccine will be administered assuming the following criteria are met:

- No active uncontrolled infection
- Platelet count  $> 20,000/\text{mm}^3$ \*
- Hemoglobin  $> 8 \text{ g/dL}$ \*
- AST/ALT, total bilirubin  $< 3$ -fold normal

\*The patient need not be RBC or platelet-transfusion independent but must be able to sustain a Hgb  $> 8 \text{ g/dL}$  or platelet  $> 20,000$  with transfusion

At the post-transplant vaccination screen, patients will be evaluated for eligibility to proceed with vaccination. The post-transplant vaccination will not be administered to patients with Grade 2 or higher hematologic toxicities based on the National Cancer Institute Common Toxicity Criteria (NCI-CTC) for bone marrow transplant studies (platelet count  $< 20,000/\text{mm}^3$ , and hemoglobin  $< 8 \text{ g/dL}$ ).

Patients who do not meet the eligibility for vaccination will be evaluated weekly until the blood counts improve to Grade 1 or better (platelet count > 20,000/mm<sup>3</sup> and hemoglobin >8g/dL) at which time they will receive the vaccine.

The cell lines used for the vaccine preparation have undergone extensive regulatory testing and have been determined to be sterile and free of mycoplasma and viral contamination. The vaccine clinical lots were manufactured and released by the Cell Processing and Gene Therapy Facility, a cGMP compliant facility, at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. Dr. Borrello will submit the manufacturing and product release data to the FDA prior to initiating the study. The vaccine cells are irradiated prior to cryopreservation and stored frozen in the vapor phase of liquid nitrogen until the day of use. The details of production, irradiation, freezing, and preparation for administration are discussed in detail in the Investigational New Drug (IND) submission. Equal numbers ( $5 \times 10^7$  each) of the myeloma cell lines H929 and U266 cells will be combined with the bystander cell line K562/GM-CSF ( $5 \times 10^6$ ) to prepare the vaccine. Vaccine cells frozen in an injectable formulation Pentaspan® (10% Pentastarch in 0.9% sodium chloride with 2% human serum albumin and 5% DMSO) will be thawed on the day of vaccination in a 37°C water bath, mixed, and then taken up into five labeled syringes prior to administration. The syringes are kept on ice until administration. Injections should be started within 60 minutes of the thaw.

Toxicities associated with the intravenous administration of Pentaspan® include circulatory overload, abnormalities of coagulation (prolonged prothrombin, partial thromboplastin and clotting times), and hypersensitivity reactions characterized by wheezing, urticaria, hypotension, and anaphylactic/anaphylactoid reactions. Additionally, patients who are allergic to corn can also develop hypersensitivity to Pentaspan®.

Each vaccination will consist of five total intra-dermal injections, two each in the right and left anterior upper thighs, and one in the non-dominant upper arm (unless contraindicated). In the event that the specified limb is contraindicated, the dominant arm may be used. Vaccine injections sites shall be at least 5 cm at needle entry from the nearest neighbor. The approximate volume of each vaccination injection is approximately 0.3-0.4ml.

A lidocaine based topical anesthetic (may include but is not limited to EMLA or ELA-MAX) cream will be recommended being applied to each injection site approximately 1-2 hours prior to vaccination to diminish the discomfort associated with the intra-dermal injections. "Approximately" allows for the topical anesthetic to be placed up to two and a half hours prior to the vaccine administration. The topical anesthetic is optional and participants are allowed to



decline its use. Self-application of the topical anesthetic is allowed after the research participant receives instruction. If the research participant is non-compliant with topical anesthetic administration instructions, the topical anesthetic will be applied by the research nurse. If the subject is allergic to lidocaine, the topical anesthetic will not be used. All research participants will be seen in the oncology outpatient center for vaccine administration and monitoring.

Each dose will be administered on an outpatient basis. The subject must be observed in the clinic for at least 30 minutes after vaccination is completed.

### **13 Observation and Evaluation After Treatment with Activated MILs**

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Follow-up evaluation of the patient will be carried out according to standard institutional guidelines for autologous stem cell transplantation. In addition, the patient will have a limited number of tests performed specifically for this protocol. Any toxicities felt to be related to the infusion of aMILs will be reported as adverse events.

**See Appendix E, *Patient Study Calendar***

#### **13.1 Evaluations from Day 4 through Day 28 after Autologous Stem Cell Transplant**

**The following tests will be performed daily from Day 4 until the day of confirmed engraftment for both neutrophils and platelets (See section 13.7.1 for definition of engraftment)**

- Daily CBC ( $\pm$  24 hours) with differential and platelet count (differential will only be performed if the total WBC  $>$  100/ $\mu$ l as per standard institutional practice).

**The following tests will be performed on Days 7, 14, and 21**

- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin
- Study blood draw (100ml) in heparinized syringes
- Tiger top tube 10 cc

**The following will be performed on Day 28 ( $\pm 2$  days)**

- Bone marrow biopsy and aspirate (collect 20ml for research purposes)
- M-protein, serum and urine:
  - Protein electrophoresis
  - Immunofixation (if no measurable M-spike in serum or urine)
  - Freelite (sensitive assay for kappa and lambda light chains) only if most recent immunofixation result showed no evidence of M-protein
- Quantitative immunoglobulins (IgG, IgM, IgA)
- Clinical assessment to include disease response/progression/relapse (the clinical assessment can be performed up to 10 days after the laboratory evaluation)
- Concurrent medication documentation
- Bone turnover markers: Serum C telopeptide levels and bAlkaline phosphatase and osteocalcin
- Green top tubes (total of 20 cc)
- Study blood draw (100ml) in heparinized syringes
- Tiger top tube 10 cc

**13.2 Evaluations from Day 60-360 after Autologous Stem Cell Transplant**

The patients will be evaluated post transplant on day  $60 \pm 7$  days, day  $180 \pm 14$  days, day  $300 \pm 14$  days, and day  $360 \pm 30$  days. The day 300 visit will only be required of patients randomized to the vaccine arm of the protocol.

- Clinical assessment: review of systems, ECOG performance status, vital signs
- CBC with differential and platelet count
- Serum chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, ALT, AST, total bilirubin, alkaline phosphatase, albumin
- M-protein, serum and urine:
  - Protein electrophoresis
  - Immunofixation (if no measurable M-spike in serum or urine)

- Freelite (sensitive assay for kappa and lambda light chains) only if most recent immunofixation result showed no evidence of M-protein
- Quantitative immunoglobulins (IgG, IgM, IgA)
- Disease response/progression/relapse assessment (the clinical assessment can be performed up to 10 days after the laboratory evaluation)
- Concurrent medication documentation
- Adverse event documentation until progression/relapse or Day 360, whichever occurs first
- Bone marrow aspirate and biopsy. The bone marrow examination will be performed on days 28,  $60 \pm 7$  days,  $180 \pm 14$  days and  $360 \pm 30$  days. Additional bone marrow (at least 20ml) will be obtained to assess for T cell subsets, as well as evaluation of tumor specificity. The samples need to be collected prior to vaccination.
- Study Bloods:
  - Green top tubes (total of 20 cc)
  - Study blood draw (100ml) in heparinized syringes
  - Tiger top tube 10 cc Bone marrow turnover markers: serum C telopeptide and bAlkaline phosphatase and osteocalcin These tests will not be performed on day 300.

### 13.3 Management of Progressive Disease

Patients with disease progression/relapse shall have this documented. Once the patient has progressed/relapsed, treatment will not be dictated or limited by the protocol, but the patient will continue to be followed for survival for five years from Day 0.

It should be noted that since the purpose of this study is to examine the anti-tumor efficacy of aMILs combined with a myeloma-specific vaccine, **no post-transplant maintenance therapy is permitted while on the study.**

### 13.4 Discontinuation of Evaluations after Treatment

There are several reasons that a patient may be discontinued from the post-treatment protocol evaluations. These include, but are not limited to:

- Patient decision to withdraw from the study
- Protocol non-compliance

- Patient lost to follow-up
- Activated MILs product failed to meet specifications
- Activated MILs product was not infused

The reason for discontinuation of the protocol evaluations will be documented. The patient should continue to be followed annually for survival when possible.

As described in Section 2.2.2, patients will be monitored for progression/relapse on Days 60, 180, and 360, and as clinically indicated. Following one year follow-up, patients will be followed annually for the next four years.

### 13.5 Contraindicated Medications

There are some medications that may have adverse effects on the activity of activated MILs. These medications and/or therapies should **NOT** be given to patients at any time during the entire study unless indicated in the protocol or to treat life-threatening conditions. Patients should not be placed on any maintenance therapy following transplant. Contraindicated medications and therapies include:

- Corticosteroids (e.g., hydrocortisone, prednisone, prednisolone [56], dexamthasone (Decadron), etc.). Inhaled steroids for the use of allergic rhinitis or pulmonary disease are allowed and not contraindicated.
- Thalidomide
- Interferon
- Growth factors, interleukins, or other cytokines (except filgrastim as outlined in the protocol, or erythropoietin)
- Cytotoxic agents (except cyclophosphamide for stem cell mobilization and high-dose melphalan as outlined in the protocol)
- Other immunosuppressive drugs
- Other experimental therapies
- Radiation therapy

**NOTE: Corticosteroids treatment may have an adverse effect on the activity of activated MILS. Each patient's hospital and clinic chart will be labeled "NO STEROID MEDICATIONS EXCEPT FOR EMERGENCIES" on the cover.**

After disease progression, use of corticosteroids and other medications are not dictated by the protocol.

## **14 Risk and Toxicity Assessment**

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If the Principal Investigator determines that an adverse event is sufficiently severe to remove the patient from the study, an assessment should be performed and the patient should be given appropriate treatment under medical supervision.

Patients will be subject to the risks associated with high-dose chemotherapy and autologous stem cell transplant. Because autologous stem cell transplantation is considered a standard treatment option in multiple myeloma, this section will focus on the risks associated with the infusion of activated MILs, as well as unforeseen adverse events that could result from the interaction of activated MILs with the stem cell transplantation therapy.

### **14.1 Risks of Venous Access**

Complications associated with venous access for leukapheresis of stem cells and the infusion of stem cells and activated MILs include discomfort, bruising, and/or bleeding at the catheter insertion site, thrombosis of the accessed vessel, and infection. Complications from placement of a central line required for the high-dose chemotherapy and stem cell transplantation procedure and activated MILs include increased risk and severity of the above complications, as well as pneumothorax.

### **14.2 Risks of MILs Bone Marrow Collection**

The patients will undergo a standard bedside bone marrow aspiration with sedation and sterile technique to collect the marrow infiltrating lymphocytes for subsequent T cell activation. There is a potential risk of excessive pain that will be handled with analgesia as needed. There is also the possibility of local and/or systemic infection.

### **14.3 Potential Microbial Contamination of the Activated MILs**

There is the potential that microorganisms inadvertently introduced during cell collection and processing could cause an infection in the patient following infusion of the activated MILs. All precautions to maintain sterility will be taken. Cultures will be obtained prior to and after completing the T cell activation process.

The activated MILs product will not be released until 14-day sterility testing is complete. In the event that there is a sterility failure during final product release testing, the activated MILs will not be administered.

#### **14.4 Potential Toxicity of Storage Solutions**

##### **14.4.1 Dimethyl Sulfoxide (DMSO)**

aMILs products and the Myeloma Vaccine are cryopreserved in a solution containing approximately 5% DMSO. DMSO is a cryoprotectant used to store a variety of infused cell products including hematopoietic stem cells and bone marrow. After cells are thawed, DMSO contained in the freezing solution is infused along with the cells. Toxicity associated with DMSO infusion is usually limited to minor changes in heart rate, blood pressure, fever, chills, sweats, nausea, vomiting and headaches. These are usually self-limited, lasting no more than a few hours. The most notable effect is a garlicky odor due to exhalation of a DMSO metabolite that may last up to one day. More severe adverse effects include severe acute allergic reactions including anaphylactoid reactions, pulmonary embolism, respiratory failure, renal failure, cardiac arrhythmias, seizures, and death.[57, 58] These side effects are rarely observed and occur primarily in patients receiving much higher volumes of cryopreserved cell products than being infused in the present study. Nevertheless, patients will be carefully monitored for any adverse effects as outlined in Section 0, *Adverse Events*. Any adverse events should be documented and recorded on the appropriate Case Report Form.

##### **14.4.2 Human Serum Albumin (HSA)**

aMILs products and the Myeloma Vaccine are formulated in a cryoprotectant containing 2% human serum albumin. Albumin solutions are FDA approved for volume resuscitation (treatment of hypovolemia, shock, and burns). Although hypersensitivity reactions are possible, reactions are not anticipated to the small amount of albumin administered.

##### **14.4.3 6% Hetastarch**

aMILs are formulated in a cryoprotectant containing 6% Hetastarch in 0.9% sodium chloride. Toxicities associated with the intravenous administration of 6% Hetastarch include circulatory overload, abnormalities of coagulation (prolonged prothrombin, partial thromboplastin and clotting times), and hypersensitivity reactions characterized by wheezing, urticaria, hypotension, and anaphylactic/anaphylactoid reactions.

#### 14.4.4 Pentaspan®

The cell lines used to prepare the Myeloma Vaccine are formulated in a cryoprotectant containing Pentaspan® (10% pentastarch in 0.9% sodium chloride). Toxicities associated with the intravenous administration of Pentaspan® include circulatory overload, abnormalities of coagulation (prolonged prothrombin, partial thromboplastin and clotting times), and hypersensitivity reactions characterized by wheezing, urticaria, hypotension, and anaphylactic/anaphylactoid reactions. Additionally, patients who are allergic to corn can also develop hypersensitivity to Pentaspan®.

#### 14.5 Potential Adverse Effects Associated with the Allogeneic Myeloma Vaccine

The vaccine will consist of two allogeneic myeloma cell lines, H929 and U266 admixed with a GM-CSF - secreting bystander cell line, K562/GM-CSF. GM-CSF secreting vaccines have been administered to over 300 patients and toxicities have been limited to localized vaccine site reactions involving erythema and tenderness. Rarely, systemic reactions have been reported such as low-grade fevers and Grover's syndrome (personal communication, Dr. Elizabeth Jaffee).

These two allogeneic cell lines have never been used in a vaccine formulation prior to this study. However, considering the vast experience at Johns Hopkins alone with other allogeneic vaccine formulations: breast, prostate and pancreas cancer, and the absence of any rate limiting toxicities, we cannot anticipate any possible toxicities from this vaccine formulation.

#### 14.6 Potential Adverse Effects Associated with Activated MILs

Previous clinical trials using CD3xCD28 bead-activated and expanded peripheral blood T cells were described above. Eighty-nine HIV patients have been treated, with the most common toxicities being fevers, chills, fatigue, rash, sinusitis, asthenia, headaches, and nausea. [59-62] Grade 3 and 4 toxicities were predominantly associated with IL-2, which was infused in some of the patients in these trials. T cells activated with CD3xCD28 beads have also been administered to patients undergoing autologous stem cell transplantation for relapsed or refractory Non-Hodgkin's lymphoma.[51] T cells were collected prior to high-dose chemotherapy. Fourteen days following peripheral blood stem cell infusion, activated and expanded T cells were administered. Three patients were treated at a median cell dose of  $0.4 \times 10^9$ , twelve patients were treated at a median cell dose of  $1.6 \times 10^9$ , and two patients were treated with a median cell dose of  $9.8 \times 10^9$ . The latter two patients experienced infusion related toxicities including transient fever, dyspnea, rigors and pulmonary edema (one patient).

It is possible that activated T cells could target the patient's own tissues, resulting in autoimmune disorders. However, this was thought to be due to high-dose IL-2, a known cause of this disorder, which was given with the cell therapy. Autoimmune disease has not been observed in previous studies using CD3xCD28 bead-activated T cells. Considering that in this case, the lymphocytes are obtained from the marrow microenvironment, it is possible that an immune response could develop towards normal hematopoietic elements. For this reason, hematologic engraftment will be closely monitored throughout the trial.

#### **14.7 Potential Adverse Events Associated with Autologous Stem Cell Transplant**

Patients will be at risk of all of the complications associated with the autologous stem cell transplant procedure. The patients will be signing a separate consent form for the autologous stem cell transplant utilizing the standard of care protocol currently in use in our institution. This will be done per institutional practice by the attending physician on the Bone Marrow Transplant Service. The Investigator is responsible for discussing these risks in detail with the patient. The following is a brief overview of the major risks associated with autologous stem cell transplantation.

##### **14.7.1 Peripheral Blood Stem Cell Mobilization Chemotherapy Regimen**

Patients receive cyclophosphamide chemotherapy and filgrastim to mobilize peripheral blood stem cells for collection. Cyclophosphamide can cause alopecia, nausea, vomiting, bladder irritation and hemorrhage, and myelosuppression including anemia, leukopenia, and thrombocytopenia. Patients are at risk of bleeding and infection.

##### **14.7.2 Administration of Filgrastim (G-CSF)**

Filgrastim is given in the peripheral blood stem cell mobilization regimen and can cause significant bone pain in many patients.

##### **14.7.3 Melphalan**

High doses of melphalan can cause nausea, vomiting, fatigue, anemia, and alopecia. Patients are at significant risk of serious and life-threatening infections during the neutropenic period, which usually lasts for approximately two weeks. Patients will likely require red blood cell and platelet transfusions, both of which are associated with infectious risks such as hepatitis and HIV.

##### **14.7.4 Leukapheresis**

Patients will require leukapheresis to collect the peripheral blood stem cells



#### 14.7.5 Hematopoietic Stem Cell Infusion

The hematopoietic stem cells may become contaminated with microbes during their collection or processing. Infusion of hematopoietic stem cells contaminated with microbes such as bacteria could cause an infection. The hematopoietic stem cells are stored in DMSO. Similar to the administration of activated MILs, the DMSO is infused along with the cells.

#### 14.8 Risks of Interactions Between Activated MILs, Tumor Vaccination & Autologous Stem Cell Transplantation

It is possible there may be unforeseen interactions between the activated MILs and high-dose chemotherapy followed by autologous stem cell transplantation. Such interactions could cause, for example, delays in neutrophil or platelet recovery, the development of autoimmune disease, or exacerbations of known transplant toxicities, such as mucositis. Patients will be monitored closely for hematopoietic engraftment, and adverse events.

### **15 Adverse Events**

#### **15.1 Case Report Form Reporting**

Only the adverse events (AEs) occurring during the study from the time of bone marrow collections of MILs and myeloma vaccine through Day 360 that are felt to be related to the experimental products (collection or infusion of aMILs as well as vaccine) and not a result of the standard and accepted toxicity associated with autologous stem cell transplantation will be documented and recorded on the AE Case Report Form. AEs will not be collected past Day 360 or after the patient progresses. AEs that occur after Day 360 or disease progression/relapse would likely reflect the disease process, and not the activated MILs or vaccination.

Clinically significant laboratory results considered to be possibly related to aMILs and not related to autologous stem cell transplant will also be recorded as AEs. For purposes of this study, clinical significance is defined as any laboratory result that is outside the range of normal, as defined by each laboratory's recorded reference range and is deemed clinically significant by the Principal Investigator.

Adverse events and con-meds will be assessed on an on-going basis during patient treatment. Serious adverse events and unexpected adverse events will be reported per section 15.4. All adverse events documentation related to the infusion of aMILs will continue until resolution of those events.

Reporting will include a description, onset and resolution date, severity, and assessment of relatedness to activated MILs, vaccination or other suspect cause.

## 15.2 Grading of Adverse Events and Toxicities

AEs and toxicities will be graded using the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) (<http://ctep.cancer.gov/reporting/ctc.html>). AEs that are not included in the NCI Common Toxicity Criteria will be graded as follows:

- Mild (Grade 1) – near the lowest intensity (or within the lower one-third) typically seen with the observed sign or symptom
- Moderate (Grade 2) – average intensity typically seen with the observed sign or symptom
- Severe (Grade 3) – near the highest intensity (or within the top third) typically seen with the observed sign or symptom
- Life-threatening or disabling (Grade 4) – any AE that places the patient, in the view of the investigator, at immediate risk of death from the reaction as it occurred. It does not refer to an event which hypothetically might have caused death if it were more severe.
- Fatal (Grade 5)

**Note:** These terms are used to describe intensity of a specific event; the event itself may be of relatively minor medical significance (such as severe headache).

## 15.3 Attribution of Causality

The association or relationship of the AEs to activated MILs or myeloma vaccine will be defined according to the NCI Common Toxicity Criteria guidelines as follows:

- Definite – The AE is *clearly related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of drug
- Probable – The AE is *likely related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug, that is confirmed by stopping

or reducing the dosage of the drug, and that could be reasonably explained by the known characteristics of the subject's clinical state

- Possible – The AE *may be related* to the study product, e.g., an event that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug, but that could readily have been produced by a number of other factors
- Unlikely – The AE is *doubtfully related* to the study product
- Unrelated – The AE is *clearly not related* to the study product
- Not applicable – The AE occurred prior to administration of the study product
- Unknown – No evaluation for causality can be made

#### **15.4 Adverse Events Requiring Immediate Notification of IRB, IBC, FDA and NIH RAC**

The Principal Investigator is obligated to report certain AEs to the FDA (see 21 CFR, part 312.32) associated with the activated MILs or vaccination that are “serious” or “unexpected” (see definitions below):

- Serious Adverse Event - any adverse experience which is fatal or life-threatening, permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose
- Unexpected Adverse Event - any adverse experience that is not identified in nature, severity or frequency in the current Investigator Brochure

The Principal Investigator will notify the Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC) as per institutional guidelines.

##### **15.4.1 Adverse Drug Reaction Reporting**

**TOXICITY WILL BE SCORED USING CTCAE VERSION 3.0 FOR TOXICITY AND ADVERSE EVENT REPORTING. A COPY OF THE CTC VERSION 3.0 CAN BE DOWNLOADED FROM THE CTEP HOMEPAGE (<http://ctep.info.nih.gov>). ALL APPROPRIATE TREATMENT AREAS HAVE ACCESS TO A COPY OF THE CTCAE VERSION 3.0. ALL ADVERSE CLINICAL EXPERIENCES, WHETHER OBSERVED BY THE STUDY STAFF OR REPORTED BY THE PATIENT, MUST BE RECORDED, WITH DETAILS ABOUT THE DURATION AND INTENSITY OF EACH EPISODE, THE ACTION TAKEN WITH RESPECT TO THE TEST DRUG, AND THE PATIENT'S OUTCOME. THE**

**INVESTIGATOR MUST EVALUATE EACH ADVERSE EXPERIENCE FOR ITS RELATIONSHIP TO THE TEST DRUG AND FOR ITS SERIOUSNESS.**

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15.4.2 Investigator Reporting Responsibilities

**The conduct of the study will comply with all FDA safety reporting requirements.**

**All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The investigator is responsible for evaluating all adverse events to determine whether criteria for “serious” and “unexpected” as defined above are present.**

15.4.3 Report of Adverse Events to the Institutional Review Board and Institutional Biosafety Committee

The Principal Investigator is required to notify his/her Institutional Review Board (IRB) of events that meet the definition of “unanticipated involving the risks to participants and others” within 10 working days of observing or learning of the serious and unexpected adverse event. The report should identify all safety reports previously filed with the IND concerning a similar adverse reaction, and should analyze the significance of the adverse reaction in light of the previous, similar reports.

15.4.4 Investigator Reporting to the FDA and RAC

Adverse drug reactions that are **serious, unlisted/unexpected, and at least possibly associated to the drug**, and that have not previously been reported should be reported promptly to the Food and Drug Administration (FDA) in writing on a Med Watch (FDA Form #3500A) by each investigator/physician engaged in clinical research. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

7 Calendar-Day Telephone or Fax Report

The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product(s): activated myeloma infiltrating lymphocytes and/or myeloma

vaccine. An unexpected adverse event is one that is not already described in the Investigator Brochure or the approved product insert. Such reports are to be telephoned or faxed to the FDA and within 7 calendar days of first learning of the event. Each telephone call or fax transmission should be directed to the FDA product review division for the Center for Biologics Evaluation and Research (This will be identified on the FDA letter assigning the IND # 14117), which is responsible for the review of the IND.

#### 15 Calendar-Day Written Report

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of activated marrow infiltrating lymphocytes and myeloma cellular vaccine. An unexpected adverse event is one that is not already described in the reference safety information.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

#### **The address of the Food and Drug Administration is:**

Center for Biologics Evaluation and Research  
Food and Drug Administration  
HFM-99, Room 200  
1401 Rockville Pike  
Rockville, MD 20852-1448

#### **The address of the NIH RAC is:**

Recombinant DNA Advisory Committee  
NIH Office of Biotechnology Activities  
National Institutes of Health  
6705 Rockledge Drive, Suite 750  
Bethesda, MD 20892-7985

## **15.5 Definitions of Response (Blade' Criteria)**

Definitions of response are adapted from the combined criteria developed for patients with multiple myeloma treated by high-dose chemotherapy and hematopoietic stem cell transplantation.[63] If confirmation of a measurement is required, the date of response/progression/relapse will be the date at which the M-protein measurement first meets the required criterion. M-protein measurements by serum protein electrophoresis (SPEP) will take precedence over quantitative immunoglobulins for determining response. The date of response/progression/relapse will be determined by the date when the relevant tests were obtained, and not by the date when the investigator reviews the test results.

### **Criteria for Evaluation and Endpoint Definitions**

The serum and urine M-protein levels obtained at baseline will be used as the reference baseline for calculation of increases or decreases in the M-protein level. The M-protein levels from the time of diagnosis will also be obtained, and response calculations using that value as baseline will also be performed in secondary analyses.

#### **15.5.1 Complete Response (CR)**

Requires all of the following:

- Absence of the original M-protein in serum by immunofixation, maintained for a minimum of 6 weeks. Presence of M-protein by Freelite assay does not exclude CR. The presence of oligoclonal bands consistent with oligoclonal immune reconstitution does not exclude CR. Normalization of serum concentrations of normal immunoglobulins is not required
- If a urine M-protein is present at baseline or other prior evaluation, absence of this M-protein in urine by immunofixation, maintained for a minimum of 6 weeks
- < 5% plasma cells in a bone marrow aspirate and on bone biopsy, if biopsy performed
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response
- Disappearance of any soft tissue plasmacytomas

#### **15.5.2 Near Complete Response (nCR)**

Requires all of the following:

- Absence of the original M-protein in serum but still identifiable by immunofixation, maintained for a minimum of 6 weeks. Presence of M-protein by Freelite assay does not exclude CR. The presence of oligoclonal bands consistent with oligoclonal immune reconstitution does not exclude CR.

Normalization of serum concentrations of normal immunoglobulins is not required

- If a urine M-protein is present at baseline or other prior evaluation, absence of this M-protein in urine but still present by immunofixation, maintained for a minimum of 6 weeks
- < 5% plasma cells in a bone marrow aspirate and on bone biopsy, if biopsy performed
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response
- Disappearance of any soft tissue plasmacytomas

#### 15.5.3 Very Good Partial Response (VGPR)

Requires all of the following:

- A decrease in the serum M-protein by  $\geq 90\%$ , maintained for a minimum of 6 weeks
- If a urine M-protein is present at baseline or other prior evaluation, absence of this protein by immunofixation, maintained for a minimum of 6 weeks
- < 5% plasma cells in a bone marrow aspirate and on bone biopsy, if biopsy performed
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response
- Disappearance of any soft tissue plasmacytomas

#### 15.5.4 Partial Response (PR)

Requires all of the following:

- A decrease in the serum M-protein by  $\geq 50\%$ , maintained for a minimum of 6 weeks
- If a urine M-protein is present at baseline or other prior evaluation, a  $\geq 90\%$  reduction in this protein, or a decrease to less than 200 mg in a 24 hour urine collection. This response must be maintained for a minimum of 6 weeks
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response
- Decrease of  $\geq 50\%$  in the size of any soft tissue plasmacytomas (by radiography or clinical examination)

#### 15.5.5 Minimal Response (MR)

Requires all of the following:

- A decrease in the serum M-protein by  $\geq 25\%$ , maintained for a minimum of 6 weeks

- If a urine M-protein is present at baseline or other prior evaluation, a  $\geq 50\%$  reduction in this protein in a 24 hour urine collection. This response must be maintained for a minimum of 6 weeks
- No increase in the size or number of lytic bone lesions on skeletal x-rays, if performed. Development of a compression fracture does not exclude response
- Decrease of  $\geq 25\%$  in the size of any soft tissue plasmacytomas (by radiography or clinical examination)

#### 15.5.6 Stable Disease

- Not meeting the criteria of CR, VGPR, PR, MR, progression, or relapse from CR

#### 15.5.7 Relapse from CR

Requires one or more of the following:

- Reappearance of serum or urinary M-protein on immunofixation or routine electrophoresis, confirmed by at least one further investigation, and excluding oligoclonal immune reconstitution
- $\geq 5\%$  plasma cells in a bone marrow aspirate or on bone biopsy
- Development of new lytic bone lesions or soft tissue plasmacytomas or definite increase in the size of residual bone lesions. Development of a compression fracture does not exclude continued response and may not indicate progression
- Development of hypercalcemia (corrected serum calcium  $> 11.5$  mg/dL or 2.8 mmol/L) not attributable to any other cause

#### 15.5.8 Progression

For patients not in CR. Requires one or more of the following:

- A  $> 25\%$  increase in the level of serum M-protein, which must also be an absolute increase of at least 5 g/L and confirmed by at least one repeat investigation.
- A  $> 25\%$  increase in the 24 hour urinary light chain excretion, which must also be an absolute increase of at least 200 mg/24 hours and confirmed by at least one repeated investigation
- A  $> 25\%$  increase in plasma cells in a bone marrow aspirate or on biopsy, which must also be an absolute increase of at least 10%
- Definite increase in the size of existing bone lesions or soft tissue plasmacytomas
- Development of new bone lesions or soft tissue plasmacytomas at any time since baseline. Development of a compression fracture does not exclude continued response and may not indicate progression



- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.8 mmol/L) at any time since baseline, which is not attributable to any other cause. (Corrected serum calcium is calculated by adding 0.8 mg/dL to the measured serum calcium for every 1 g/dL that the serum albumin falls below 4.0 g/dL.)

Reference level for increase in M-protein, plasma cells, or lesion sizes is the lowest level documented since (and including) baseline evaluation.

## **15.6 Survival Endpoints**

### **15.6.1 Overall Survival**

Measured as the time from Day 0 (day of stem cell infusion) to death from any cause. This will be monitored annually for up to 4 years.

During this annual visit, the following will occur for the patients that opt to come here for their follow-up. Alternatively, survival will be determined by the medical records obtained from the locally treating physician:

- Disease response/progression/relapse assessment (the clinical assessment can be performed up to 10 days after the laboratory evaluation)
- 100ml heparinized syringes and 20 mls total in green top tubes of blood will be collected from the patient for immune monitoring assays.
- Bone marrow biopsy and aspirate will be performed. 20ml of marrow will be collected for immune monitoring.

### **15.6.2 Progression-Free Survival**

Measured as the time from initial enrollment to progression/relapse of disease or death from any cause, whichever occurs first.

## **15.7 Definitions of Engraftment and Graft Failure**

### **15.7.1 Engraftment**

Neutrophil engraftment is defined as the day post-transplant when the absolute neutrophil count is > 500/mm<sup>3</sup> for two consecutive days post-transplant (note: bands as well as neutrophils are included in calculations). The first day on which this criterion is met is considered the day of neutrophil engraftment.

Platelet engraftment is defined as the first of three consecutive counts post-transplant when the platelet count is > 20,000/mm<sup>3</sup> without platelet transfusion support. The first day on which this criterion is met is considered the day of platelet engraftment.

#### 15.7.2 Failure of Engraftment

Primary graft failure is defined as failure to recover an absolute neutrophil count  $> 500/\text{mm}^3$  or platelet count  $> 20,000/\text{mm}^3$  by Day 28 post-transplant. Secondary graft failure is defined as a fall to less than these levels for three or more consecutive days after Day 28 without another cause.

## **16 Statistical Considerations**

### **Study Design**

This is a continuation study stemming from two previous early stage clinical evaluations: J0115 – autologous myeloma vaccine; and J0770 – activated MILs with regard to treatment effect, and tolerability of allogeneic myeloma cell vaccine and aMILs in this patient population. In the aMILs trial (J0770) we have observed the early results shown 5 out of 19 (26%) patients had complete response (CR) based on Blade' Criteria. The current trial intends to build a treatment regime using a modified higher dose of aMIL, adoptive immunotherapy, and concurrent with vaccination which had been tested in J0115 trial to enhance overall immune anti-tumor responses. Due to alterations in ex-vivo MILs expansion and the possibility that tumor specific vaccination could further augment anti-tumor immune responses, the study is designed to randomize patient onto either a high dose aMILs alone or a high dose aMILs + vaccine with 1:1 randomization ratio. The purpose of the randomization is not for hypothesis testing, but to have initial estimation of the CR rate for the two treatment regimens in a parallel fashion, second to the primary objective of an overall response assessment.

#### **16.1 Sample Size**

A total 32 evaluable patients will be needed in this clinical trial. If patients are registered but do not receive the activated T cells for any reason, then additional patients will be registered to ensure 32 patients are treated.

The primary clinical activity is defined as completed response based on Blade' Criteria. The study is designed to have initial estimation of the CR rate among those treated either with aMILs alone or aMILs+ the vaccine. Our early clinical experience using aMILs alone shows the CR rate of 26% compared to an estimated 20% CR from standard transplant. The total of 32 patients will yield a minimum of 40% CR rate with a lower bound of 95% confidence interval excess 20%. The 32 patients will be randomly assigned to either aMILs alone or aMILs +vaccine group with 1:1 randomization ratio stratified by the clinical status (newly diagnosed vs relapsed, ISS staging 1-2 vs. 3). The sample size justification based on precision of estimation is tabulated below:

No. of Patients	No. of patients with CR	% CR rate	95% Confidence Intervals
32	13	40%	24% -- 59%
32	16	50%	32% -- 68%
16	7	44%	20% -- 70%
16	8	50%	25% -- 75%

The clinical efficacy is defined as complete response based on Blade' Criteria. Overall complete response rate and individual treatment response rate will be estimated using the binomial distribution (exact method) with 95% confidence intervals. However, other response rates such as nCR , VGPR, PR, MR, and a overall response rate (CR, VGPR, PR, MR definition see section 13.5) at 360 days post transplant will be calculated as proportions and reported as a combined response for the 32 patients and separately by group as well.

## 16.2 Statistical Analyses/ Secondary objectives

Progression-free and overall survival:

The safety endpoints will be treatment-related mortality and the incidence of Grade 3 hematologic toxicity. Toxicity will be graded using the NCI Common Toxicity Criteria (Version 3.0; <http://ctep.cancer.gov/reporting/ctc.html>; see Appendix G). Treatment related mortality is defined as death not attributable to disease progression/relapse (and excluding the 'unrelated' and 'not applicable' categories in attribution of causality per Section 15.3) that occurs within the first 100 days following transplantation. Previous randomized studies involving high-dose chemotherapy and hematopoietic stem cell transplantation in patients with multiple myeloma have generally reported treatment related mortality in the 4-8% range, [64-66]although rates as high as 14-20% have been reported. [67, 68]Treatment related mortality in the current era is likely lower than these reported rates.

The toxicity will be monitored closely. If any patient develops Grade 4 hematologic toxicity of 3 weeks or longer that represents greater than a 50% decrease from their post-transplant screen, the trial will be suspended until appropriate analysis and discussion with the FDA and IRB has been conducted. The study will be terminated if there are one or more deaths, which are definitely or probably attributable specifically to the activated T cell therapy, as defined in Section 13.3, *Attribution of Causality*. However, given the toxicities associated with high-dose therapy and hematopoietic stem cell transplantation, attribution of death specifically to the experimental therapies may be difficult. Therefore the study will also be terminated and the treatment approach deemed not worthy of further investigation in this setting if at any time 4 patients die of treatment related causes or experience Grade 3 hematologic toxicity.

Disease progression is defined in the protocol section 15.5 Progression-free survival is defined as time from date of randomization to the date of first observation of disease progression or death due to disease progression. Overall survival is defined as the time from the date of randomization to date of death due to all causes. The Probability of progression-free survival (PFS) and overall survival (OS) will be estimated using the Kaplan-Meier method and displayed graphically. Median PFS and OS will be reported along with 95% confidence intervals. Proportion of patients who were PFS and OS at specific time of follow-up will be reported as well.

Statistical analyses of other secondary objectives such as T cell reconstitution, anti-tumor immune response, tumor-specific vaccine response, effect of aMIL on osteoclastogenesis, and Myeloma clonogenic precursors will be considered exploratory. Descriptive statistics will be provided. All data will be summarized based on nature distribution of data and presented separately by treatment group at clinical defined time points, or by primary clinical response.

The association between clinical responses and in vitro measurements of T cell laboratory correlates, including T cell proliferation to tumor and ELISPOT analysis of both peripheral as well as marrow infiltrating lymphocytes will be explored by using multivariate regression models as appropriate.

Evaluation of hematopoietic engraftment:

The mean and median time to neutrophil and platelet engraftment (absolute neutrophil count  $>500/\text{mm}^3$  and platelet  $>20,000/\text{mm}^3$ ) will be summarized among those with successful engraftment, along with the rate of primary graft failure. It should be remember that in the absence of post-transplant G-CSF, we would expect neutrophil engraftment to be slightly delayed. However, should red cell, platelet and neutrophil engraftment all show evidence of significant delay in more than 2 patients, the study will be placed on hold until a more formal evaluation can be performed.

All patients demographic information, disease, and baseline characteristics will be summarized for each of the study groups independently.

#### 16.4.1 Subject Accountability

The following data will be summarized:

- Number (%) of subjects registered
- Number (%) of subjects undergoing autologous stem cell transplant
- Number (%) of subjects who receive activated MILs

#### 16.4.3 Protocol Compliance

The registered study subject population will be described in reference to the study criteria. At a minimum, the following tabulations will be provided:

- Number of subjects with violations of inclusion/exclusion criteria
- Number of subjects with major on-study deviations or violations

## **17 Ethical, Regulatory, and Administrative Considerations**

### **17.1 Informed Consent**

The principles of informed consent are described in the Code of Federal Regulations 21 CFR, part 50.

Patients or their legal guardians must be made aware of the investigational nature of this treatment protocol, and have the possible risks, hazards and benefits of the protocol explained to them. The information that is given shall be in a language understandable to the patient. No informed consent, whether oral or written, may include any exculpatory language through which the patient is made to waive or appear to waive any of their legal rights, or releases or appears to release the Principal Investigator, the institution, or its agents from liability for negligence.

The patient, or legal guardian, must be able to comprehend the informed consent and must sign the document prior to consent signing on study. The patient will receive a copy of the respective signed consent form.

### **17.2 Institutional Review**

The principles of Institutional Review Board (IRB) are described in the Code of Federal Regulations 21 CFR, part 56.

The Principal Investigator will obtain approval for the study from the IRB. The Principal Investigator must notify the IRB within 5 days of protocol deviations in emergency situations regarding patient safety. The Principal Investigator will be responsible for obtaining annual IRB renewal through the duration of the study, or more frequently if required by the IRB. Copies of the Principal Investigator's report and copies of the IRB's continuance of approval must be maintained in the regulatory binder located at the clinical site.

### **17.3 Tissue Use for Research Purposes**

A portion of the MILs bone marrow product and/or final activated MILs product may remain unused at the conclusion of the study. Patients will have the option on the study consent form to allow this tissue to be used for further research purposes by the Principal Investigator, or to be destroyed one year after collection.

In addition, various patient tissues, including peripheral blood and bone marrow will be collected from patients during the course of the study expressly for research assays. Baseline bone marrow specimens with adequate tumor involvement will be used to develop primers required for a PCR based assay for minimal residual tumor in the final activated MILs product. Quantification of minimal residual disease will also be performed in bone marrow from patients in complete remission for whom primers can be developed. Peripheral blood and tumor containing marrow specimens may also be used in immune assays.

Results from assays performed for research purposes will not be provided to patients. At the conclusion of the study and follow-up period (five years from Day 0), some of this tissue may remain unused. Patients will have the option in the study consent form to allow this tissue to be used for further research purposes or to be destroyed.

## **18 Study Monitoring and Data Collection**

### **18.1 Study Monitoring**

This is a Level III study under the SKCCC CRO Data and Safety Monitoring Program. Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally by the principal investigator.

External monitoring will be performed by the SKCCC CRO in accordance with SKCCC and federal guidelines. Trial monitoring and reporting will be done through the Medical Expert Committee when approximately half the patients have been enrolled and upon completion of enrollment in the study.

#### **18.1.1 Completion of Case Report Forms (CRFs)**

The Principal Investigator or his designee will be responsible for completing, in a timely manner, a CRF for each patient who is registered to participate in this study. CRFs will be completed, as information becomes available.

The Principal Investigator or a sub-investigator will sign and date the indicated places on the CRF. This signature will indicate that a thorough inspection of the audited data therein has been made and will thereby certify the contents of the form.

#### **18.1.2 Cell Therapy Laboratory (CTL) Cell-Processing Facility**

The Cell Therapy Laboratory (CTL) is the processing lab for all cellular therapy products (bone marrow, vaccines, and adoptive T cell products) administered in the Cancer Center at Johns Hopkins. It operates according to Good Tissue and Manufacturing Practices and is accredited by FACT.

The results of cell processing using the activated T cell process will be recorded on CRFs or in the Batch Production Records at the Johns Hopkins CTL facility. CRFs and other study records will be reviewed in detail by the CRA, or designee, who will have access to all patient medical records, laboratory data, and other source documentation to verify the accuracy of the data recorded on these documents.

## **18.2 Maintenance of Study Documentation**

It is the responsibility of the Principal Investigator and study staff to maintain a comprehensive and centralized filing system of all study-related documentation. This filing system must be suitable for inspection at any time by the CRO or Quality Assurance (QA) designee of Johns Hopkins and the FDA. Elements should include:

- Subject Files - containing the completed patient CRFs, supporting source documentation, and a signed and dated Informed Consent
- Regulatory Binder - containing the protocol with all amendments, current Consent Form, the Investigator Brochure, clinical site logs, accountability records and laboratory documents (e.g., certification, norms/ranges, etc.)

### **18.2.1 Retention of Records**

The FDA requires that each Principal Investigator retain records for a period of two (2) years from the date of FDA approval to market the product for this indication, or two (2) years from the date that the Sponsor withdraws the application for approval.

## **18.3 Final Study Report**

Upon completion of the study, the Principal Investigator is required to submit a final study summary report to the Institution. This report must also be submitted to the IRB.

## **18.4 Investigational Product Labeling and Accountability**

### **18.4.1 Investigational Product Labeling**

aMILs products will be labeled at a minimum with the patient's name and history number, the unique product number, and the volume within the bag.

The Myeloma Vaccine will be labeled with the lot numbers of the cell lines used to prepare the vaccine in addition to the preparation date, product expiration time, and the patient's name and history number.

#### 18.4.2 Investigational Product Accountability

The Investigator should take adequate precautions, including storage of the investigational products, to prevent theft or diversion of the products into unauthorized channels of distribution. The processing of each product will be documented on a Batch Production Record. Release test results will be summarized on the Product Specification Report.



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## **20 Appendices**

### **Appendix A: Diagnostic Criteria for Multiple Myeloma**

- Major criteria:
  1. Plasmacytomas on tissue biopsy
  2. Bone marrow plasmacytosis (>30% plasma cells)
  3. Monoclonal immunoglobulin spike on serum electrophoresis IgG >3.5 g/dL or IgA >2.0 g/dL; kappa or lambda light chain excretion > 1 g/day on 24 hour urine protein electrophoresis
- Minor criteria:
  - a. Bone marrow plasmacytosis (10 to 30% plasma cells)
  - b. Monoclonal immunoglobulin present but of lesser magnitude than given under major criteria
  - c. Lytic bone lesions
  - d. Normal IgM <50 mg/dL, IgA <100 mg/dL or IgG <600 mg/dL

Any of the following sets of criteria will confirm the diagnosis of multiple myeloma

- Any two of the major criteria
- Major criterion 1 plus minor criterion b, c, or d
- Major criterion 3 plus minor criterion a or c
- Minor criterion a, b and c or a, b and d

Reference: Durie, B. G. 1986. Staging and kinetics of multiple myeloma. Semin.Oncol. 13[3], 300-309.

## Appendix B: Durie-Salmon Staging of Multiple Myeloma

### Stage I

All of the following must be present

- Hemoglobin > 10.5 g/dL or hematocrit > 32%
- Serum calcium level normal
- Low serum myeloma protein production rates as evidenced by all of the following:
  - IgG peak < 5g/dL
  - IgA peak < 3g/dL
  - Bence Jones protein < 4g/24 h
- No bone lesions

### Stage II

All patients who do not meet criteria for Stage I or III are considered to be Stage II.

### Stage III

One of the following abnormalities must be present:

- Hemoglobin < 8.5 g/dL, hematocrit < 25%
- Serum calcium > 12 mg/dL
- Very high serum or urine myeloma protein production rates as evidenced by one or more of the following:
  - IgG peak > 7g/dL
  - IgA peak > 5g/dL
  - Bence Jones protein > 12 g/24 h
- > 3 lytic bone lesion on bone survey (bone scan not acceptable)

### Subclassification

A: Serum creatinine < 2.0 mg/dL

B: Serum creatinine  $\geq$  2.0 mg/dL

Adapted from: Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975;36:842-54.

## Appendix C: ECOG Performance Status

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self care. Totally confined to bed or chair
5	Dead

AS PUBLISHED IN AM. J. CLIN. ONCOL. (CCT) 5:649-655, 1982

## Appendix D: New York Heart Association Classification of Patients with Diseases of the Heart

### Functional Classification

- |           |   |
|-----------|---|
| Class I   | Patient with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.  |
| Class II  | Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.  |
| Class III | Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.  |
| Class IV  | Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased. |

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(New York Heart Association Criteria Committee: Diseases of the Heart and Blood Vessels: Nomenclature and Criteria for Diagnosis, 6<sup>th</sup> ed. Boston, Little, Brown, & Co. 1964)

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## Appendix E: Patient Study Calendar

	Consent Signing Eligibility	MM Vaccination	Marrow Collection	Stem Cell Mobilization	Stem Cell Collection	Day -2 (Melphalan)	Day -1 (Melphalan)	Day 0 (Stem Cell Infusion)	Therapy Week 1							Follow-up										
									Day 1	Day 2	Day 3 aMILs Infusion*	Day 4	Day 5	Day 6 <sup>a</sup>	Day 7 <sup>a</sup>	Day 14	Day 21 Vaccine	Day 28 (±2 days)	Day 60 (±7 days) Vaccine	Day 180 (±30 days) Vaccine	Day 300 (±30 days) Vaccine <sup>12</sup>	Day 360 (±30 days)	Annually for 4 years	Off Study		
Informed Consent	X																									
Inclusion/Exclusion criteria	X																									
Clinical Assessment <sup>1</sup>	X																	X	X	X	X	X				
Bone marrow examination <sup>2</sup>			X <sup>a</sup>															X	X	X			X			
Beta2-microglobulin	X																					X	X			
HTLV1 and 2	X																									
CBC w/diff, platelets <sup>3</sup>	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Serum Chemistries <sup>3</sup>	X										X				X		X	X	X	X	X	X	X			
Serum myeloma studies <sup>4</sup>	X																X	X	X	X	X	X	X			
M-protein, urine <sup>5</sup>		X	(X)														X	X	X	X	X	X	X			
Bone Turnover Labs <sup>6</sup>	X																X	X	X	X	X	X	X			
Myeloma Vaccine <sup>7</sup>		X													X		X	X	X	X	X					
Study Blood (100ml) & Tiger top tube (10ml) <sup>8</sup>		X <sup>b,c</sup>	X										X	X	X <sup>c</sup>	X	X	X	X <sup>c</sup>	X	X	X	X			
Study Blood in Green top tubes (20ml) ) <sup>9</sup>		X <sup>d</sup>	X														X	X	X	X	X	X	X			
Disease Response/Progression																		X	X	X	X	X	X	X		
Survival Status <sup>10</sup>																		X	X	X	X	X	X	X	X	
Current Medications											X						X	X	X	X	X					
Adverse Events <sup>11</sup>			X								X															

1 Clinical assessment: history, review of systems, weight, vital signs, physical exam, ECOG. For day 28 and 60 this can be done up to 10 days later

2 Collect 20ml of marrow in a heparinized 60ml syringe. (<sup>a</sup>On day of marrow collection, patients will have 200ml of BM collected in heparinized syringes)

3 Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, AST, ALT, alk. phosph., total protein, albumin. CBC differential will only be performed if total WBC >100/ul. Labs may be drawn ± 24hrs.

4 M-protein, serum: serum protein electrophoresis & immunofixation, Serum free light assay, quantitative immunoglobulins

5 M-protein, urine: 24 hour urine collection for total protein, protein electrophoresis & immunofixation (should be obtained either with pre-transplant vaccine or at time of bone marrow collection)

6 Bone Turnover to include: Blood for C telopeptide and bAlkaline phosphatase in yellow top tube

7 Myeloma Vaccine consists of 3 cell lines admixed, irradiated and administered over 3 limbs. Pre-transplant vaccine to be administered 14 days (± 2 days)

prior to the bone marrow collection.

8 100ml of blood in heparinized syringes and 10 ml in tiger top tube will be collected at the time of bone marrow collection. <sup>b</sup> Vaccine Arm: Blood will be collected immediately before pre-transplant vaccination as well as at the time of the bone marrow collection.

<sup>c</sup> Vaccine Arm: 10 ml tiger top tube collected 2 days later.

9 20ml of blood in green top tubes will be obtained for evaluation of myeloma precursors. <sup>d</sup> Vaccine Arm: This blood can be collected either immediately before pre-transplant vaccine or at time of bone marrow collection)

10 Survival and disease status will be followed for 4 years from Day 0

11 Capture adverse events are those felt to be related to the infusion of aMILs. Standard stem cell transplant toxicities will not be captured.

12 The day 300 visit will only apply to the patients randomized to the vaccine arm. This will include a CBC with differential.

\* For aMILs infusion, patients must have T<38.5°C and room air O2 saturation of >90%.